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Environnement Canada
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Simultaneous Measurements of Trimethylamine and Dimethylamine in Fish, and Their Use for Estimating Quality of Frozen-Stored Gadoid Fillets

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CASTELL, C. H., B. SMITH, AND W. J. DYER. 1974. Simultaneous measurements of trimethylamine and dimethylamine in fish, and their use for estimating quality of frozen-stored gadoid fillets. J. Fish. Res. Board Can. 31: 383–389.

On the basis of some of the recent modifications of Dyer's colorimetric test for measuring trimethylamine (TMA) in fish, a procedure is described that gives both the TMA and the dimethylamine (DMA) contents. Measurements on 84 frozen-stored fillets of haddock, cod, pollock, cusk, and hake showed a correlation coefficient of 0.94 between DMA values obtained by the copper–dithiocarbamate test and those obtained by the picric acid procedure. The simultaneous measurements of TMA and DMA are useful in estimating the quality of frozen-stored gadoid fillets, the TMA value indicating the extent of microbial spoilage before the muscle was frozen and the DMA value the extent of deterioration through action of tissue enzymes during frozen storage.

CASTELL, C. H., B. SMITH, AND W. J. DYER. 1974. Simultaneous measurements of trimethylamine and dimethylamine in fish, and their use for estimating quality of frozen-stored gadoid fillets. J. Fish. Res. Board Can. 31: 383–389.

Utilisant les récentes modifications du test colorimétrique de Dyer pour mesurer la triméthylamine (TMA) dans le poisson, nous décrivons une technique qui donne simultanément la teneur en TMA et en diméthylamine (DMA). Des mesures effectuées sur 84 filets entreposés congelés d'aiglefin, morue, goberge, brosme et merluche démontrent un coefficient de corrélation de 0.94 entre les valeurs de DMA obtenues par le test du cuivre–dithiocarbonate et les valeurs correspondantes obtenues par la méthode de l'acide picrique. Les mesures simultanées de la TMA et DMA pourraient être utiles dans l'estimation de la qualité des filets de gadoïdes entreposés congelés: la valeur de la TMA indique l'étendue de la détérioration microbienne avant congélation du muscle, et la valeur de la DMA indiquant l'étendue de la détérioration produite par action des enzymes tissulaires durant l'entreposage réfrigéré.

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HASHIMOTO and Okaichi (1957) found that the accuracy of Dyer's (1945, 1950, 1959) colorimetric test for trimethylamine could be improved by substituting KOH in place of K_2CO_3 as the alkalizing solution. Tozawa et al. (1970) suggested that the KOH reduced the interference caused by any dimethylamine that may be present. The KOH plays a more important role than merely freeing the amine salts: it actually interferes with the formation of DMA-picrate but has little or no effect on the formation of TMA picrate.

Although the substitution of KOH for K_2CO_3 reduces the development of color from DMA, it does not entirely eliminate it. Tozawa et al. (1970, 1971) have shown that when KOH is used in this procedure it still produces sufficient color to give readable OD values on the spectrophotometer. In other words, changing the alkalies simply changes the proportion of TMA and DMA that react to give a color with the picric acid. This suggests that an equation might be developed from which the amounts of both TMA and DMA could be determined by using a combination of the results from Dyer's procedure and Tozawa's modification of it.

The experiments described in this paper were carried out to determine both the possibility and the practicability of using the picric acid procedures for measuring both TMA and DMA in fish muscle.

General Procedures

The procedures for preparing the reagents and carrying out the test and calculations for TMA determinations by the picric acid method have already been described (Dyer 1945, 1950, and 1959) and these were carried out except for the following minor modifications: Extract the muscle by blending it for 45 s with an equal weight of 10% trichloracetic acid, and let stand for 30 min with occasional shakings before filtering. To 0.5 and/or 1 ml of the filtered extract add 1 ml of formalin and make solutions up to 5 ml with water, using screw-topped tubes with lightly fitting plastic lids. After shaking vigorously 50 times, allow solutions to sit until the aqueous and toluene layers have separated (about 2 or 3 min). Remove approximately 5 ml from the toluene layer to a tube containing about 0.5 g of Na_2SO_4; shake and let settle. Transfer 5 ml of the dried toluene to a cuvette and add 5 ml of picric acid reagent and, after mixing, read optical density at 410 nm using a Beckman DU spectrophotometer.

For the alkali solutions, Baker's reagent grade, anhydrous potassium carbonate, and Fisher's certified potassium hydroxide (pellets) were used. Because of variations in the water content of the KOH pellets we prepared a saturated solution containing 51.7% (w/w) KOH, from which we prepared other solutions with the desired KOH concentrations.

Experimental

Extractabilities of TMA and DMA with toluene — A considerable difference in the extractabilities of TMA and DMA from aqueous solutions by toluene was shown by the following experiment. Two amine solutions were prepared, one containing 0.01 mg TMA-N/ml and the other 0.02 mg of DMA-N/ml. These were subjected to the usual procedures of Dyer's picric acid test, up to the stage where the 7 ml of toluene were removed from the upper layer in the extraction tubes. A special effort was then made to remove all the remaining toluene from above the aqueous solutions, which were then subjected to a series of nine more similar extractions, and picric acid reagent was added to the toluene from each of the successive extractions. The resulting OD values (Fig. 1) show that during the first extraction the toluene removed approximately 80% of the TMA but only 20% of the DMA. It required at least six successive extractions to remove 80% of the DMA. This is an important point that

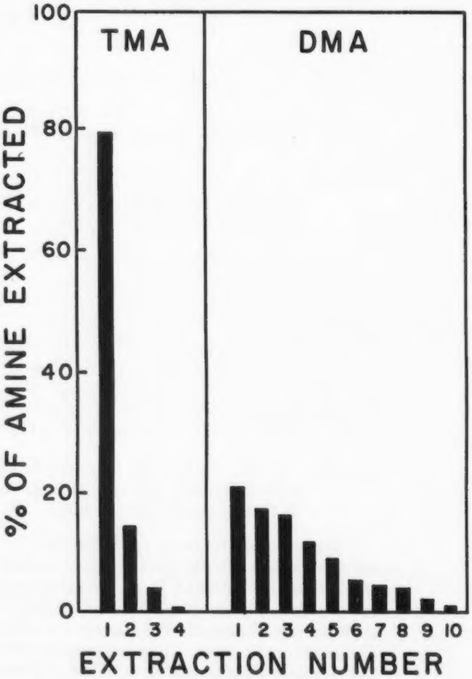

FIG. 1. Percentages of the total TMA and DMA in successive toluene extractions from solutions initially containing 0.01 mg of TMA and 0.02 mg DMA. The extraction tubes were shaken by hand and the alkali solution used was 50% K_2CO_3. The amounts of TMA and DMA shown in the curves in Fig. 1, 2, and 3 are milligrams of amine nitrogen/sample.

must be kept in mind in the development of a procedure for extracting DMA from an aqueous solution by toluene.

Amount of shaking in extraction of DMA — That the amount of shaking of the extraction tubes was more critical with DMA than with TMA was shown by the following experiment comparing five procedures for shaking the tubes. Some were shaken by hand and others with a mechanical super-mix-touch shaker. Ten tubes containing 0.02 mg DMA-N were used for each of the procedures and the average OD values were as follows:

Method of shaking	Avg OD values
By hand, 40 times slowly	0.070
By hand, 40 times vigorously	0.115
By hand, 200 times vigorously	0.185
By shaker, 2 min at full speed	0.190
By shaker, 5 min at full speed	0.230

Similar tests with various mechanical shakers showed that, although they were all better than shaking by hand, some were more efficient than others. The most satisfactory results were obtained with a "Cole-Palmer multipurpose rotator," which handled 16 tubes at a time. Using this rotator–shaker, with samples containing 0.01 mg DMA-N/tube, OD values were obtained for shaking times ranging from 0.5 s to 60 min (lowest curve in Fig. 2). It required between 12 and 15 min to establish an equilibrium in the distribution of the DMA between the aqueous and toluene layers. This figure also shows the effect of increasing the amount of DMA in the extraction tubes for shaking periods of 1–15 min.

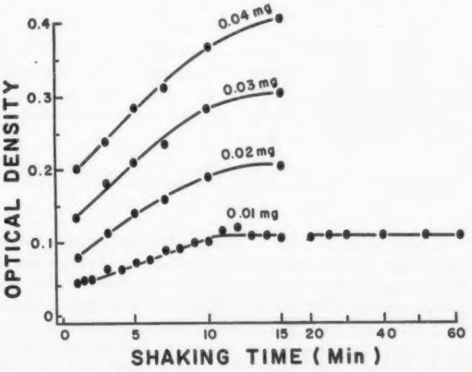

FIG. 2. Optical densities of DMA picrates when four strengths of DMA in aqueous solutions were extracted with toluene for various periods in a Cole-Parmer rotator–shaker running at 50 rpm and 50% K_2CO_3 was used as the alkali solution.

Under certain conditions the time elapsing between the termination of the shaking and the removal of the toluene from the tubes had a considerable effect on the amount of DMA taken up by the toluene. A solution of DMA was divided into 18 portions. Nine of these were shaken in the rotator–shaker for 15 min and then left to stand for various periods of time before removing the toluene. The other nine samples were shaken 40 times by hand and then left to stand for various periods. Holding periods from 1 min to 2 h did not alter the OD values of the samples that had been shaken 15 min in the rotator–shaker. But the holding time did have an effect on the hand-shaken samples:

Holding time	OD value
0.5	.070
2	.080
5	.095
10	.105
20	.110
30	.120
60	.125
80	.138
240	.165

This indicates that the holding period will influence only the uptake of DMA by the toluene if at the end of the shaking a distribution equilibrium has not been established.

Concentrations of alkali solutions — Previous workers have not given much data to support their selection of the concentrations of the alkali solutions to be used in the various modifications of the picric acid test for TMA. Dyer (1945) used a 50% (w/w) solution of K_2CO_3, which is very near to being a saturated solution. Hashimoto and Okaichi (1957) recommended that this be replaced by a 25% solution of KOH. Murray and Gibson (1972) found that higher readings were obtained if the concentration of KOH was increased from 25 to 45%. They also reported that the calibration curve for TMA against optical density was more nearly linear and more readily reproducible with the higher concentration of KOH, although this is not evident from the data in their figure and table.

We examined the effects of a much wider range (Fig. 3) of alkali concentrations, with both TMA and DMA, than the 25 and 45% solutions of KOH and the 50% solution of K_2CO_3 used by other workers. With a constant concentration of TMA and increasing concentrations of K_2CO_3 through a range of 5–50%, the relation of color intensity of the picrate to the K_2CO_3 concentration was linear; but with KOH the relation was linear up to approximately 30% and above this point the slopes leveled off with both TMA and DMA. Also, before the picric acid reagent was added, the amine solutions in the higher concentrations of KOH were slightly yellow; this did not occur in the lower concentrations that produced the linear relationship with the color intensities. These observations suggest

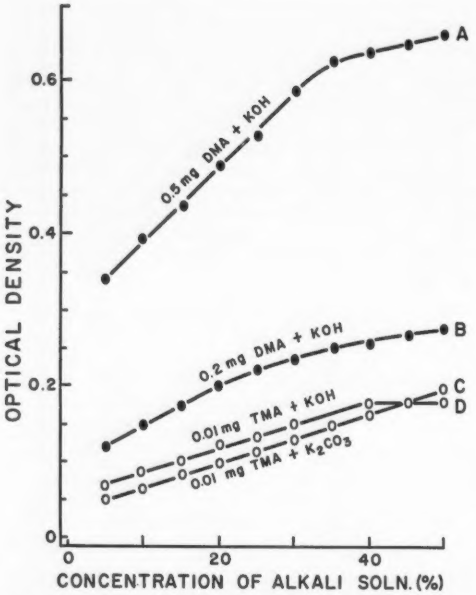

FIG. 3. Optical densities of TMA and DMA picrates when various concentrations of KOH and K_2CO_3 solutions were used as the alkalizing agents.

that, until more has been learned about the reactions taking place between the amines and the higher concentrations of KOH, the 25% solution used by Hashimoto and Okaichi (1957) is preferable to the 45% solution recommended by Murray and Gibson (1972).

Absorption curve for DMA-picrate — Dyer (1950) and Murray and Gibson (1972) found that the maximum absorption for TMA picrate occurred at 410 nm. A similar curve, with a maximum at 410 nm, was found for DMA picrate.

Standard curves for TMA and DMA — Though standard curves for both TMA and DMA using KOH and K_2CO_3 have already been developed for calculating the amounts of these amines in fish extracts by the picric acid procedures (Tozawa et al. 1970), our observations suggested that the curves for DMA would be altered by a more complete extraction of this amine in the toluene. With this in mind a fresh set of standard curves (Fig. 4) were developed, based on tests in which all the extraction tubes were shaken for 15 min on the rotator–shaker. In another set of tests the OD values were determined on 30 samples containing 0.01 mg of DMA-N using 50% K_2CO_3 and 16 samples containing 0.1 mg DMA-N using 25% KOH. The average values were: DMA-N with K_2CO_3, 0.108 (SD ±0.004); DMA-N with KOH, 0.069 (SD ±0.035). These additional results are in close agreement with the corresponding DMA concentrations shown in Fig. 4. A comparison of our results with those of Tozawa et al. for DMA indicated that the more vigorous shaking resulted in a 23% increase in OD values using K_2CO_3 and a 37% increase using KOH.

Calculations for obtaining DMA and TMA values from picric acid tests — From the standard curves, the equivalent color development (OD) can be obtained for TMA and DMA using the two alkali solutions:

Amine	Alk.	Soln.	OD for 0.01 mg amine nitrogen	OD ratio DMA: TMA	Color from DMA as % of color from TMA
DMA	50%	K_2CO_3	0.108	54:100	54
TMA			0.199		
DMA	25%	KOH	0.007	5.01:100	5.0
TMA			0.138		

Therefore, K_2CO_3 amine value = TMA + 0.54 DMA (A) and KOH amine value = TMA + 0.05 DMA (B) subtracting (B) from (A)

$$\text{DMA} = \frac{K_2CO_3 \text{ amine value} - \text{KOH amine value}}{0.54\text{–}0.05}$$

$$= \frac{K_2CO_3 \text{ amine value} - \text{KOH amine value}}{0.49}$$

For all practical purposes the DMA value equals the difference between the K_2CO_3 and KOH amine values, divided by 0.5. To obtain the TMA value, equation (B) is multiplied by $\frac{0.54}{0.05}$ and subtracted from equation (A), giving: K_2CO_3 amine value – 10.8 KOH amine value = TMA – (10.8 TMA); 9.8 TMA = 10.8 KOH amine value – K_2CO_3 amine value; TMA = 1.1 KOH amine value – 0.10 K_2CO_3 amine value.

These equations apply only when the more efficient method for amine extraction has been used. As a matter of interest, the corresponding equation for obtaining the approximate DMA value, using the procedure in which the extraction tubes were shaken 50 times by hand, was found to be:

$$\text{DMA} = \frac{K_2CO_3 \text{ amine value} - \text{KOH amine value}}{0.15}$$

Comparison of DMA values obtained by the picric acid and dithiocarbamate procedures — Comparisons of the two procedures were made by determining the DMA values of 84 fillets of frozen cod, haddock, cusk, and hake that had undergone a wide range of deterioration after they had been placed in frozen storage. A TCA extract was made from each fillet and the two DMA determinations were made on the same extract from each fillet. The results (Fig. 5) showed a correlation coefficient of 0.94 between the values obtained by the picric acid tests, using the equation developed in the previous section, and the corresponding values using the copper–dithiocarbamate procedure (Dyer and Mounsey 1945).

Unreliability of picric acid tests when applied to badly spoiled unfrozen fillets — The equations for calculating DMA and TMA from the results of the picric acid tests are not reliable when applied to badly spoiled, iced, or chilled fillets. As it is unlikely that fish in these late stages of decomposition would be encountered under normal commercial conditions, this is of academic interest only. An example was encountered in tests made on 36 chilled cod, cusk, and hake fillets in various stages of deterioration, ranging from fresh to offensively putrid. Their DMA values were determined using the copper–dithiocarbamate method and the picric acid tests in the same way as for the frozen fillets. With these unfrozen fish the two sets of DMA values showed fairly close correlation for the fresh fillets and those undergoing the early stages of spoilage; but once the fish had reached the stage where they smelled putrid, the "DMA values" derived from the picric acid tests became much greater than those from the copper–dithiocarbamate test. Probably in the putrid fillets some substance other than TMA or DMA is present that reacts with the picric acid, giving the higher values; this interference is likely due to the formation of higher nonvolatile amines during the later stages of spoilage, as found by Dyer and Mounsey (1945).

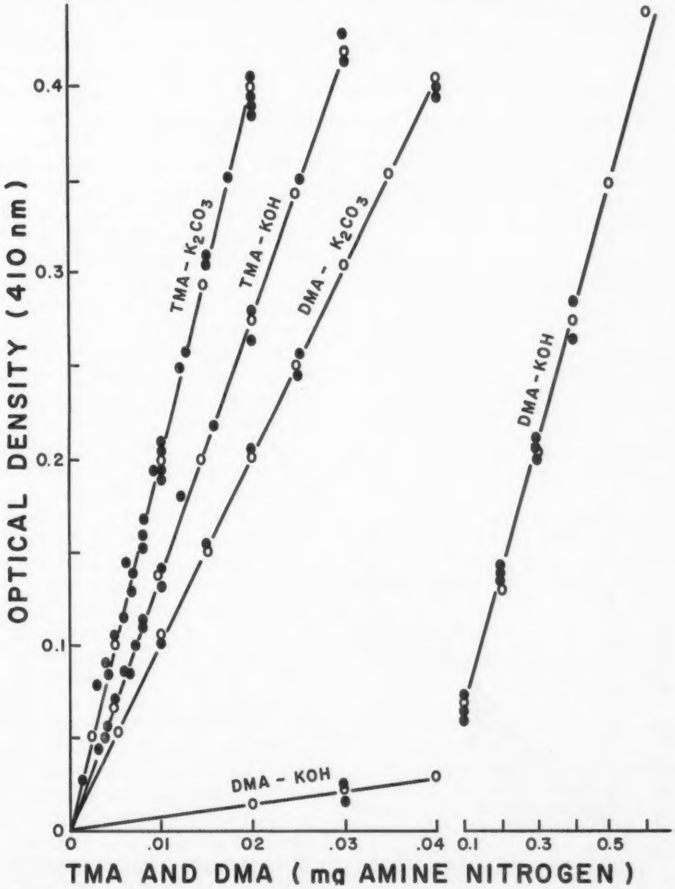

FIG. 4. Standard curves for TMA and DMA picrates when 25% KOH and 50% K_2CO_3 were used as the alkalizing agents and the extraction tubes were shaken for 15 min at 50 rpm in the rotator–shaker. Open circles, from one set of tests carried out at the same time; closed circles, from a number of tests carried out at other times.

Discussion

These findings are useful to those involved in routine assessment and control of quality of many species of marine fish. The picric acid test carried out in duplicate on the same fish extract, using K_2CO_3 as the alkali in one test and KOH as the alkali in the other, provides a relatively simple, routine method of measuring both TMA and DMA.

For some species of marine fish, including the gadoids, the amount of TMAO that has been reduced to TMA provides a useful indication of the overall bacterial activity that results in the production of undesirable odors and flavors. Over the years, within certain limitations, TMA has been found to be one of the most useful criteria for following the development of spoilage in iced and chilled commercial fish from the Canadian east coast.

Recent investigations on the chemical changes taking place in stored, frozen-stored gadoid fillets (Castell et al. 1970; Castell 1971; Castell et al. 1973a; Castell et al. 1973b) indicate that DMA can be used as a measure of the deterioration that occurs during frozen storage for these fish, in much the same manner that TMA is used to measure spoilage in the iced or chilled fish. Although DMA

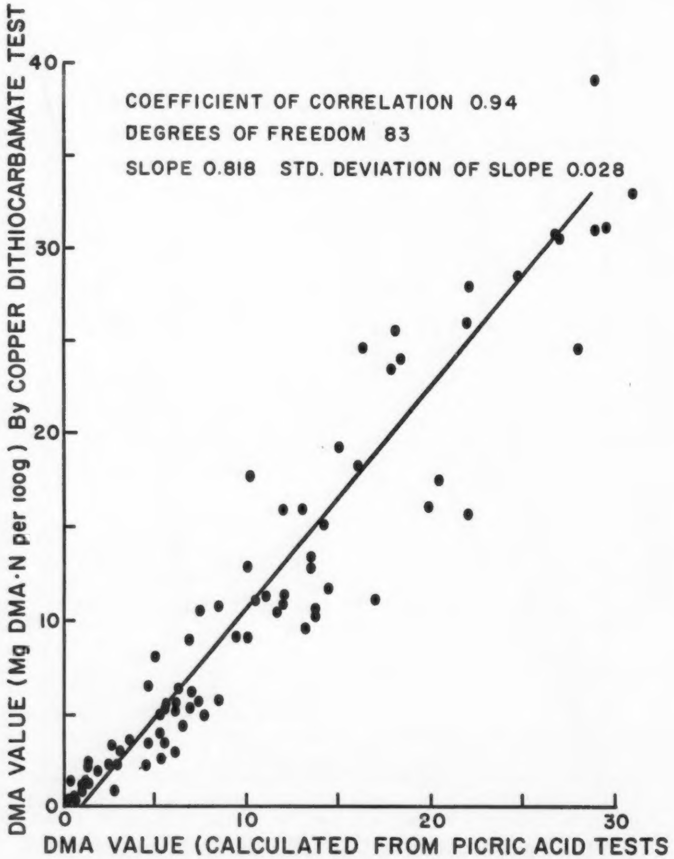

FIG. 5. Comparison of DMA values obtained with the copper dithiocarbamate test and those calculated for the same fish extracts by the picric acid procedures using K_2CO_3 and KOH as the alkali solutions. Tests made on 84 frozen-stored fillets of cod, haddock, cusk, and hake in various stages of frozen-storage deterioration.

in itself does not contribute significantly to the objectionable changes that take place in the frozen fish muscle, it is a useful means of monitoring these changes. The reasons for this are as follows: The enzyme that produces DMA in the muscle also produces an equivalent amount of formaldehyde. This enzyme activity is not inhibited by freezing unless the storage temperature is close to –29 C (–20 F). The combination of the formaldehyde with the proteins and other components of the muscle is one of the main factors responsible for the toughened texture and some of the other undesirable characteristics of deteriorating frozen-stored gadoid fillets. Because some of the formaldehyde is so firmly bound to the compounds with which it reacts, there is considerable difficulty in extracting it for quantitative determinations. The DMA, on the other hand, accumulates as a free salt and is more easily extracted and measured. The accumulation of DMA is the simplest means of estimating the amount of formaldehyde that has been produced (Castell et al. 1973b).

This method for simultaneous measurement of TMA and DMA is of use especially in estimating the quality of frozen-stored gadoid fillets. The TMA value is a measure of loss in quality that occurred through bacterial action before the fish were frozen, and the DMA of deterioration that occurred during frozen storage.

It is now generally agreed that the picric acid test, using KOH as the alkali, is a more acceptable method for measuring TMA in fishery products than Dyer's original procedure using K_2CO_3. However, even this modification has some limitations and may produce results that are erroneous. Although KOH reduces the formation of the colored DMA picrate, it does not eliminate it. It was shown above that, when the extraction tubes were shaken for 15 min in the rotator–shaker, DMA produced approximately 5% of the color that was produced by an equivalent amount of TMA. This means that, in extracts where TMA equals or is in excess of the DMA, the color is predominantly from the TMA. But when only DMA is present, or in extracts in which DMA is greatly in excess of TMA, one cannot be certain that the color is indicative of TMA. This very condition of high DMA values with little or no TMA is encountered in gadoid muscle that has deteriorated in frozen storage, especially with fillets of pollock, cusk, or hake. This difficulty can be overcome by carrying out the picric acid procedure with both alkali solutions and calculating the TMA value from the following equation: $\mathrm{TMA} = 1.1$ KOH amine value – 0.10 K_2CO_3 amine value.

Use of the terms "K_2 amine value" and "KO amine value" — In the past, many workers, including the authors, have used the term "TMA value" (milligrams TMA-N/100 g) in recording the results from Dyer's picric acid procedure. Without qualifying information this use of the term is inaccurate. Even when KOH has been substituted for K_2CO_3 there is nothing to ensure that all, or part, of the color development is not due to the formation of DMA picrate. It is more accurate to simply record the results of these tests as "amine values." To distinguish the results of tests in which K_2CO_3 has been used as the alkali from those in which KOH has been used, the former can be designated as "K_2-amine values" and the latter "KO-amine values."

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Effects of Interactions of Irradiance, Daylength, and Temperature on Division Rates of Three Species of Marine Unicellular Algae

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HOBSON, L. A. 1974. Effects of interactions of irradiance, daylength, and temperature on division rates of three species of marine unicellular algae. J. Fish. Res. Board Can. 31: 391–395.

Rates of light-saturated division of *Thalassiosira fluviatilis* and *Isochrysis* sp., marine unicellular algae, were regulated by temperature and daylength at 25 C, and primarily by temperature at 20 C or less. The rates for *Chroomonas salina* appeared to be regulated by temperature and daylength at temperatures between 10 and 25 C, with maximum effects of daylength occurring at temperatures of 20 and 25 C. Physiological mechanisms through which temperature and daylength interact to regulate division rates of unicellular algae are discussed.

HOBSON, L. A. 1974. Effects of interactions of irradiance, daylength, and temperature on division rates of three species of marine unicellular algae. J. Fish. Res. Board Can. 31: 391–395.

Le taux de division, sous éclairage saturé, des algues unicellulaires marines *Thalassiosira fluviatilis* et *Isochrysis* sp. est réglé par la température et la longueur du jour à 25 C, et en grande partie par la température à 20 C ou moins. Les taux, chez *Chroomonas salina*, semblent réglés par la température et la longueur du jour à des températures de 10 à 25 C, avec effet maximal de la longueur du jour entre 20 et 25 C. L'auteur examine les mécanismes physiologiques de l'interaction de la température et de la longueur du jour dans le réglage des taux de division des algues unicellulaires.

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IRRADIANCE, daylength, and temperature are three variables demonstrated in laboratory studies to be important in regulating division rates of unicellular algae. Numerous studies have been carried out to examine the response of division rates to changing values of any two of these variables with the third variable held constant at some predetermined value (e.g. Castenholz 1964; Jitts et al. 1964; Paasche 1967, 1968). Only one study is known to the author in which the effects of varying irradiance, daylength, and temperature on rates of accumulation of cell material have been investigated (Tamiya et al. 1955). These authors used the freshwater alga *Chorella ellipsoidea* as a test organism, and found that the magnitude of the irradiance just giving a light-saturated growth rate was a direct function of temperature, that growth rates increased with increasing daylength, and that the rate of this increase was temperature dependent.

The present research was designed to extend our information on effects of irradiance, daylength, and temperature on division rates of unicellular algae from freshwater to marine organisms. Experiments examining the response of division rates of *Thalassiosira fluviatilis* Hustedt (Clone Actin), *Isochrysis* sp. (Clone Iso.), and *Chroomonas salina* (Wislouch) Butcher (Clone 3C), from the culture collection at the Woods Hole Oceanographic Institution, to 48 combinations of three daylengths, four irradiances, and four temperatures were carried out from May 1971 to March 1972. The results of these experiments are reported in this paper.

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Materials and Methods

Cells were axenically cultured in 50 ml of half-strength f medium (Guillard and Ryther 1962) in Pyrex tubes (20 × 2.5 cm). Cell numbers were determined every 12, 24, or 48 h for periods ranging from 1 to 13 days depending on length of lag phases of growth. Division rates were calculated for the exponential phase of growth by the equation:

$$\mu = \frac{\log_2 C_2 - \log_2 C_1}{t_2 - t_1},$$

where μ is the growth constant in divisions per day, and C_2 and C_1 are cell concentrations in cells per milliliter at times t_2 and t_1 in days.

Stock cultures of the three species were maintained at 18 C in an incubator illuminated by cool-white fluorescent lights giving a light–dark cycle of 18 and 6 h. These cultures were used as a source of cells for experimental studies, and to determine whether or not division rates of cells systematically changed during the 8-mo experimental period. No long-term trends in division rates of cells of *T. fluviatilis* and *C. salina* were apparent (Fig. 1). However, division rates of *Isochrysis* sp. increased from 0.75–1.3 day^{-1} to 1.8 day^{-1} in February and remained high until experiments were terminated. This change in division rate was associated with a change in cell morphology from a motile to a nonmotile stage of cell structure (Parke 1949).

Experimental studies were carried out in an incubator with light–dark cycle and temperature controls, and equipped with high-output cool-white fluorescent lights giving an irradiance of 0.10 cal cm^{-2} min^{-1} at vessel surfaces. This irradiance and its directionality were determined by a radiometer from the Yellow Springs Instrument Co. Illumination of the long axis of the culture tubes was primarily direct light from the sources; only a small proportion was from light reflected off the side walls, back, and top and bottom of the incubator. The irradiance in the incubator was later determined with a Star Pyranometer (Model 28 AM 100) from the Kahl Scientific Co., on a spherical surface. These values were in close agreement. Irradiance within cultures was regulated by appropriate amounts of cheesecloth wrapped around the tubes. Extinction of light by the cheesecloth was determined by placing the cloth between the light source and radiometer. Rate of extinction was not linearly related to number of cloth layers.

Cells were aseptically transferred from stock cultures to experimental cultures, which were kept at daylengths of 6, 12, or 18 h and temperatures of 10, 15, 20, or 25 C, and at maximum irradiance until cells reached the exponential phase of growth. Then the cells were used as inocula for experimental cultures kept at the same daylength and temperature but at irradiances of 0.007, 0.014, 0.040, or 0.10 cal cm^{-2} min^{-1}. Light energy received by cultures in 24 h was calculated by computing the irradiation given to cultures during the light phase, and expressing these results in calories received by cultures per day.

The order in which the combinations of experimental conditions were tested was selected at random. Duplicates of experimental cultures were used to give information about variability in measurements of division rate. Variability was calculated with a one-way analysis of variance and was expressed as the standard deviation, at $P = 0.05$, of the pooled measurements. These standard deviations were not related to values of division rates, and were ±0.19 div day^{-1} for *Isochrysis* sp., ±0.21 div day^{-1} for *C. salina*, and ±0.38 div day^{-1} for *T. fluviatilis*.

Results

Most results (Fig. 2–4) showed that largest effects of changes in daylength and in temperature on division rates (μ) occurred when division rates were light-saturated. These responses of division rates are illustrated by approximate values of maximum division rates (μ_{max}) at light saturation (Table 1). In general, μ_{max} of all organisms increased either as daylength was increased from 6 to 18 h and temperature was held constant, or as temperature was increased from 10 to 25 C and daylength was held constant. However, μ_{max} of *Isochrysis* sp. decreased as temperature increased from 20 to 25 C when cells were exposed to a daylength of 18 h.

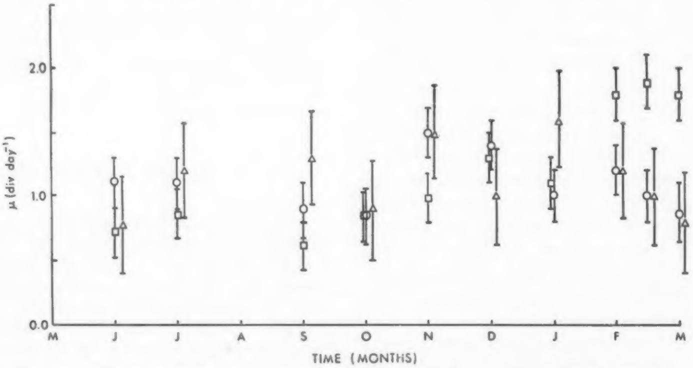

FIG. 1. Mean division rates during exponential phase of growth of *Thalassiosira fluviatilis* (△), *Isochrysis* sp. (□), and *Chroomonas salina* (○) in stock cultures during 10 mo. Vertical bars indicate standard deviations ($P = 0.05$) of pooled measurements for two replicates.

Effects of changes in daylength on μ_{max} as a function of temperature were estimated by subtracting the minimum μ_{max} from the maximum μ_{max} occurring at a given temperature in a daylength interval of 6–18 h. Results of this estimation (Table 2) indicated that changes in daylength caused larger changes in μ_{max} at high than at low temperatures. Effects of changes in temperature on μ_{max} as functions of temperature and daylength were studied with the use of temperature coefficients (Q_{10}) of μ_{max}. Coefficients were calculated for 5 C temperature intervals with the equation:

$$\ln Q_{10} = \frac{10 \ln (\mu_{max_2}/\mu_{max_1})}{T_2 - T_1},$$

where μ_{max_1} and μ_{max_2} are maximum division rates in div day^{-1} at temperatures T_1 and T_2 (Table 3). Values for *T. fluviatilis* in the temperature interval of 10–15 C and at all tested daylengths could not be calculated because cells did not divide at 10 C during the 2-wk growth period. In the temperature interval of 10–15 C, coefficients for *Isochrysis* sp. and *C. salina* varied between 1.1 and 5.1. Most coefficients varied between 1.2 and 2.6 in the temperature interval of 15–20 C. In this interval, a coefficient for *C. salina* with a 6-h daylength could not be calculated because cells grew at identical rates at 15 and 20 C. Most coefficients varied between 1.2 and 4.8 in the temperature interval of 20–25 C. The coefficient for *Isochrysis* sp. was less than 1 when cells grew with an 18-h daylength because division rates decreased as temperature increased from 20 to 25 C.

Most results (Fig. 2–4) showed that effects of daylength on μ were nonexistent or were within the experimental error at irradiations less than required

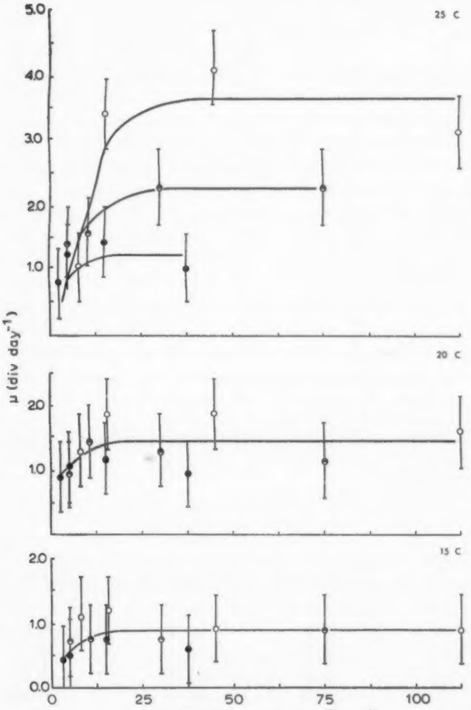

FIG. 2. Mean division rates during exponential phase of growth of *T. fluviatilis* at different irradiations and temperatures with 6-h (●), 12-h (◒), and 18-h (○) daylengths. Vertical bars as in Fig. 1.

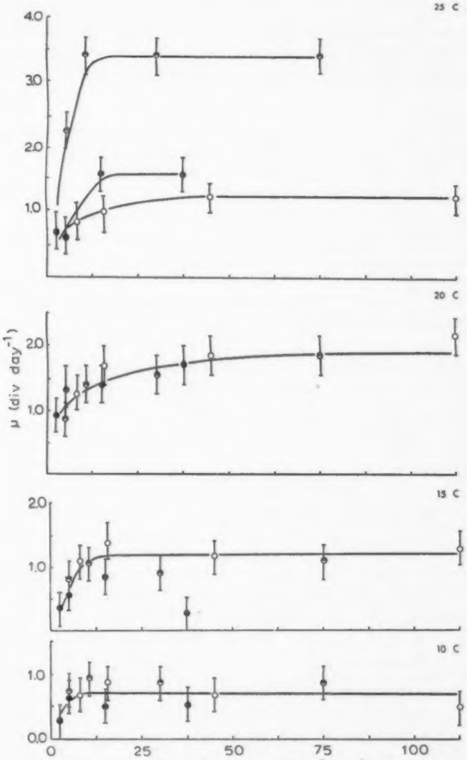

FIG. 3. Mean division rates during exponential phase of growth of *Isochrysis* sp. at different irradiations and temperatures with 6-h (●), 12-h (◒), and 18-h (○) daylengths. Vertical bars as in Fig. 1.

to saturate μ, and at temperatures less than or equal to 20 C. An exception to this observation was provided by *C. salina* cells when cultured at 20 C: division rates of these cells when cultured with 6-h daylengths and irradiations greater than 12 cal cm^{-2} day^{-1} were less than those of cells cultured with 12-h and 18-h daylengths (Fig. 4). When cells were cultured at 25 C, daylength did affect μ of the three species at irradiations less than required for saturation of μ: cells of *Isochrysis* sp. divided most rapidly when cultured with 12 h of light (Fig. 3), those of *T. fluviatilis* when cultured with 18 h of light (Fig. 2), and those of *C. salina* when cultured with 12 or 18 h of light (Fig. 4).

Discussion

These results and those of Tamiya et al. (1955) indicate that the maximum effect of daylength on either division or growth rates of unicellular algae, whether marine or freshwater, occurs at the highest tested temperatures (20–25 C) and at irradiations giving saturated division or saturated growth rates.

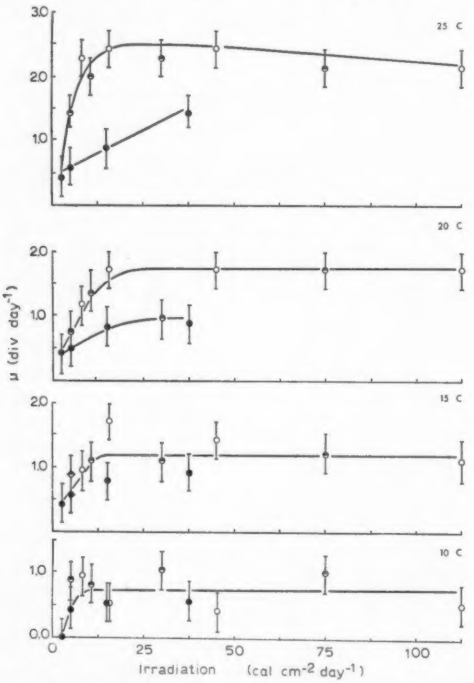

FIG. 4. Mean division rates during exponential phase of growth of *C. salina* at different irradiations and temperatures with 6-h (●), 12-h (◒), and 18-h (○) daylengths. Vertical bars as in Fig. 1.

The mechanism(s) by which daylength interacts with temperature to regulate division rates of unicellular algae is not well known. Daylength may regulate division rates when irradiation is not sufficient to saturate cell division by regulating the amount of light energy received by cells in 24-h periods (Terborgh and Thimann 1964). However, when irradiation of cells is sufficient to saturate division rates, the regulating effect of daylength must be mediated through interactions between daylength and unknown cellular mechanisms. Under these conditions, division rates of cells are

TABLE 1. Values of maximum division rates (μ_{max}) at light saturation for various temperatures and daylengths.

Temp (*C*)	Daylength (*h*)	Div day^{-1} *T. fluviatilis*	*Isochrysis* sp.	*C. salina*
10	6	U[a]	0.58	0.54
	12	U[a]	0.93	0.97
	18	U[a]	0.70	0.62
15	6	0.70	0.87	0.87
	12	0.80	1.0	1.2
	18	1.1	1.3	1.4
20	6	1.1	1.4	0.87
	12	1.3	1.6	1.3
	18	1.7	1.9	1.7
25	6	1.2	1.6	1.4
	12	2.3	3.5	2.2
	18	3.6	1.2	2.3

[a] Undetectable division rate.

TABLE 2. Changes in maximum division rates (μ_{max}) at four temperatures with changes in daylength from 6 to 18 h.

Temp (*C*)	Div day^{-1} *T. fluviatilis*	*Isochrysis* sp.	*C. salina*
10	0.0	+0.35[a]	+0.43[a]
15	+0.40	+0.43	+0.53
20	+0.60	+0.50	+0.83
25	+2.4	–2.3[b]	+0.90

[a] Change in μ_{max} with a change in daylength from 6 to 12 h.

[b] Change in μ_{max} with a change in daylength from 12 to 18 h.

TABLE 3. Temperature coefficients (Q_{10}) of maximum division rates (μ_{max}) for various temperature intervals and daylengths.

Temp interval (*C*)	Daylength (*h*)	*T. fluviatilis*	*Isochrysis* sp.	*C. salina*
10–15	6	–	2.3	2.6
	12	–	1.1	1.4
	18	–	3.4	5.1
15–20	6	2.5	2.6	–
	12	2.6	2.6	1.2
	18	2.4	2.1	1.5
20–25	6	1.2	1.3	2.6
	12	3.1	4.8	2.9
	18	4.3	0.4	1.8

probably limited by activities of cellular enzymes, and these activities are regulated by temperature. That this regulation occurs is substantiated by temperature coefficients of cell division.

Temperature coefficients for photosynthetic fixation of CO_2 by algae and by pea chloroplasts (Baldry et al. 1966) were compared to coefficients calculated from the present study, because no other coefficients for division rates of algae were found in the literature. This comparison assumes that division rates and fixation of CO_2 have approximately the same temperature coefficients. The coefficients compiled and determined by Baldry et al. (1966) varied between about 1.9 and 4.8, 1.5 and 2.5, and 1.3 and 2.1 in the temperature intervals of 10–15 C, 15–20 C, and 20–25 C, respectively. These ranges include many of the coefficients calculated in the present study for temperatures equal to or less than 20 C, suggesting that increases in temperature primarily were responsible for increases in division rates. However, many coefficients for *T. fluviatilis* and *Isochrysis* sp. in the temperature interval of 20–25 C fell outside of the range 1.3–2.1, suggesting that interactions of daylength and temperature were responsible for changes in division rates. Apparently daylength either augmented or inhibited effects of temperature on division rates of these organisms as the temperature increased. Perhaps daylength, in addition to temperature, can regulate the activity of cellular enzymes, particularly at high temperatures.

Acknowledgments

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Infectious Pancreatic Necrosis Virus Occurrence at a Hatchery in Alberta[1]

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YAMAMOTO, T. 1974. Infectious pancreatic necrosis virus occurrence at a hatchery in Alberta. J. Fish. Res. Board Can. 31: 397–402.

The presence of infectious pancreatic necrosis (IPN) virus in brook (*Salvelinus fontinalis*) and rainbow (*Salmo gairdneri*) trout was identified and further investigated. A high incidence of virus was found by virological examination of kidney tissue from yearling and older trout. By comparison, brook trout had a much higher incidence of virus than rainbow trout.

The virus was isolated most frequently from the kidneys of both species of trout. These findings indicate that the examinations of peritoneal washes and feces is not adequate for the detection of IPN carrier fish.

YAMAMOTO, T. 1974. Infectious pancreatic necrosis virus occurrence at a hatchery in Alberta. J. Fish. Res. Board Can. 31: 397–402.

L'auteur a décelé la présence du virus de la pancréatique infectieuse nécrosante (PIN) chez l'omble de fontaine (*Salvelinus fontinalis*) et la truite arc-en-ciel (*Salmo gairdneri*) et a poussé plus loin ses recherches. L'examen du tissu rénal de poissons de moins d'un an et de poissons plus âgés démontre que le virus est d'occurrence très commune. Il se rencontre beaucoup plus fréquemment chez l'omble de fontaine que chez la truite arc-en-ciel.

Le virus a été isolé le plus souvent du rein des deux espèces. Ces observations démontrent que l'examen des liquides de lavage du péritoine et des matières fécales n'est pas une méthode adéquate de détection des poissons porteurs de la PIN.

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THE acute disease of salmonids, infectious pancreatic necrosis (IPN), first described in 1941 is now known to exist in North America, Europe, and Japan (Wolf 1971). The infectious nature of the disease was experimentally demonstrated by Snieszko et al. (1959) and Wood et al. (1955) and the viral nature was first demonstrated by Wolf et al. (1960).

The virus is experimentally known to cause an acute disease with high mortalities in young salmonids (Wolf et al. 1961; Wolf and Quimby 1971), but in older fish there is a reduction in mortalities, and disease can be absent (Snieszko et al. 1959). Many of the fish that recover are known to be persistent carriers of the virus and shed the virus into water in combination with reproductive products during spawning (Wolf et al. 1961; Wolf et al. 1968; Billi and Wolf 1969). The virus can thus be transmitted from one generation to another through contaminated eggs. Workers have also indicated that there may be a vertical transmission via the egg (Wolf et al. 1963).

The Maligne River Hatchery located in Jasper National Park, Alberta, Canada has, at least on one occasion and possibly more than once, been thought to have imported contaminated eggs from Eastern Canada and possibly from the United States. IPN virus was first isolated in 1964 from diseased hatchery brook trout by Dr R. R. Rucker. However, the virus was suspected to be present as early as 1955 by Dr K. Wolf. The virus was more recently isolated and identified by Dr R. MacKelvie in 1970 and by Dr L. A. McDermott from yearling brook trout in 1971 (personal communication). Because of these isolations a more complete analysis of IPN virus distribution at the hatchery was undertaken during the summer of 1972.

Materials and Methods

METHOD OF SAMPLING FISH SPECIMENS

Throughout the summer the majority of lots of fish from the hatchery were examined at least once. Because of the importance of carrier brood stock as a potential source for the spread of virus from one generation to

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[1] Part of this investigation was presented in February, 1973 at a meeting of the Great Plains Fish Workers Association in Calgary, Alta. and in June, 1973 at a meeting of the Canadian Society of Microbiologists in Edmonton, Alta.

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another, they were examined first. Living or freshly killed and iced specimens were transported to the Edmonton laboratory where they were examined virologically.

Aseptic removal of organ samples was begun within 24 h. The method for removing and pooling organs differed slightly with different lots. Some organs were assayed separately; in other lots the organs of each animal were pooled and assayed as a single sample.

VIRUS ASSAY

Standard virological procedures were used to remove tissues for homogenization. The homogenized tissues were diluted to approximately 20% with sterile 0.85% saline and centrifuged at 12,000 XG for 10 min at 4 C. The virus containing supernatants were either filtered through Swinny filters containing 45 nm pore size filters and a prefilter or treated with antibiotics (200 IU/ml penicillin, 200 μg/ml streptomycin, and 200 μg/ml kanamycin) at room temperature for 24 h. This method was a modification of that reported by Amend and Pietsch (1972). Although the efficiencies of the filter method and the antibiotic method were not directly compared, the antibiotic method was the method of choice.

TISSUE CULTURE FOR VIRUS ISOLATION

Two different cell lines were used: the CHSE-214 chinook embryo (obtained from J. L. Fryer) and RTG-2 cell lines ATCC-CCL-55. The cultures were grown at 22 C with Minimal Eagle's Medium (Gibco, powder) with penicillin (100 IU/ml), streptomycin (100 μg/ml) and 10% fetal calf serum. Cells for diagnostic isolation were treated with 0.25% trypsin, centrifuged, and seeded into 16 × 125 mm culture tubes in 1-ml aliquots, and maintained at room temperature.

When the tube cultures approached a monolayer, they were inoculated with 0.1 ml of inoculum in duplicate and incubated at 18 C. The presence of a virus was detected by rounding and destruction of cells. Tube cultures were considered negative for virus if the cytopathic effect did not appear within 3 wk. Cultures showing toxicity were repassed into fresh cultures to detect the possible presence of virus.

ETHER SENSITIVITY

A virus sample harvested from infected CHSE-214 cells known to contain 10^{-5} TCD_{50}/ml (tissue culture limiting dilution assay) was shaken with an equal volume of ether for 5 min at 22 C in a vortex tube shaker, allowed to separate, and the water layer containing the virus assayed for infectivity in CHSE-214 tube cultures. A second aliquot of virus sample treated in an identical manner but without the ether was also assayed for infectivity.

LIGHT MICROSCOPY

Infected and uninfected cells of both RTG-2 and CHSE-214 were photographed in culture tubes as unfixed and unstained preparations after several days incubation at low multiplicity of infection. The hematoxylin–eosin stained cultures were grown on coverslips in Leighton tubes. Infection of 10 × 22 mm coverslip cultures was with approximately 10^{-6} TCD_{50}. These cultures were fixed 24 h after infection.

ELECTRON MICROSCOPY

The virus isolated from fish and grown in tissue culture was examined morphologically with the phosphotungstate negative contrast stain and after shadowing with palladium. The dimensions of the virus were determined using 92-nm diameter latex particles as a size standard.

Virus was concentrated by differential centrifugation or by banding on CsCl in a Spinco L-2B centrifuge with a SW-50.1 rotor at 32,000 rpm (95,000 × G) for 5 h.

Thin sections of experimentally infected culture cells and naturally infected fish kidneys were prepared and examined for virus according to the method described earlier (Yamamoto 1969).

SEROLOGIC IDENTIFICATION

Antiserum against IPN virus was obtained from J. L. Fryer, Department of Microbiology, Oregon State University, Corvallis, Oreg. The antiserum was diluted 1/200 and an equal volume mixed with the virus suspension diluted to contain 100 TCD_{50}/ml. After shaking, the antiserum–virus mixture was allowed to stand for 1 h at 22 C before being titrated for virus activity in CHSE-214 tube cultures.

Results

EFFECT OF VIRUS ON TISSUE CULTURE

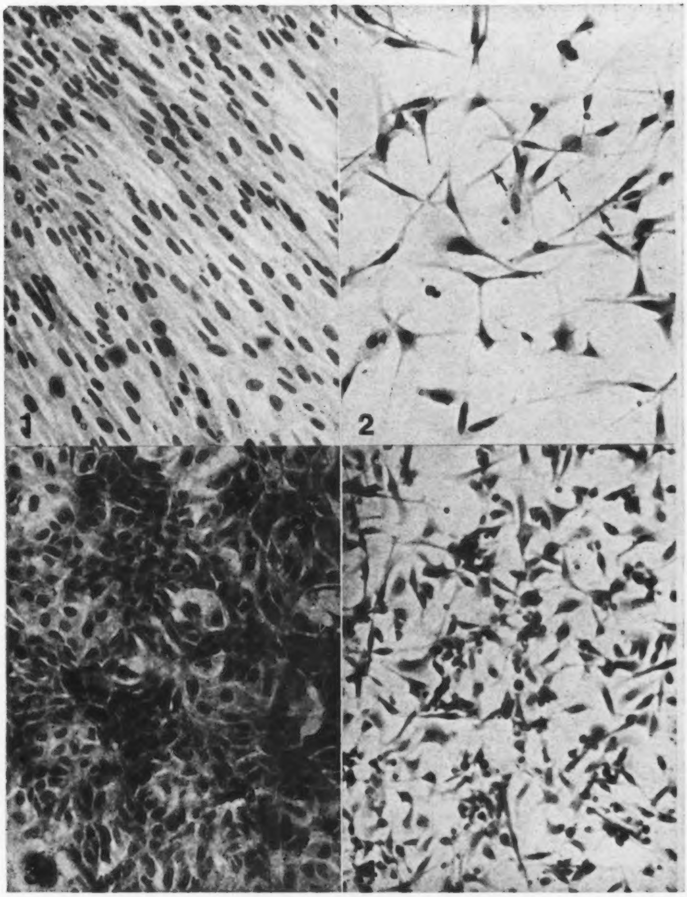
Some of the kidney specimens of yearling brook trout produced CPE as early as 24 h, progressing to gross CPE by 48 h, whereas other specimens, particularly those from rainbow trout brood stock, had very low titers and CPE did not appear for a week or more after inoculation.

For isolation purposes both cell types appeared to be equally sensitive, but CPE could be more readily detected in CHSE-214 cells early after inoculation.

Hematoxylin–eosin stained preparations of IPN virus-infected RTG-2 and CHSE-214 cells in Fig. 1–4 show the typical appearance of infected cultures. Even at high magnification there were no detectable inclusions in the nucleus or in the cytoplasm. A characteristic feature seen in RTG-2 cells infected with IPN virus was the enlarged areas of the filamentous portion of the cells giving them a characteristic feathery appearance.

IDENTIFICATION OF VIRUS

Two separate neutralization tests using IPN antiserum showed partial neutralization indicating that the virus was a strain of IPN but probably



FIGURES 1–4.

FIG. 1. Uninfected cultures of RTG-2 cells stained with hematoxylin–eosin. The typical parallel arrangement of the cells is evident; FIG. 2. Cultures of RTG-2 cells infected with IPN virus and stained with hematoxylin-eosin 24 h after infection. The orderly appearance of the cells has been destroyed and the cell filaments often show enlarged areas (indicated by arrows) but inclusions are not observable; FIG. 3. Uninfected CHSE-214 cells stained with hematoxylin–eosin. The characteristic feature is that of a typical epithelial type morphology; FIG. 4. IPN virus infected CHSE-214 cells showing the rounding type of CPE and pycnotic degeneration of cells.

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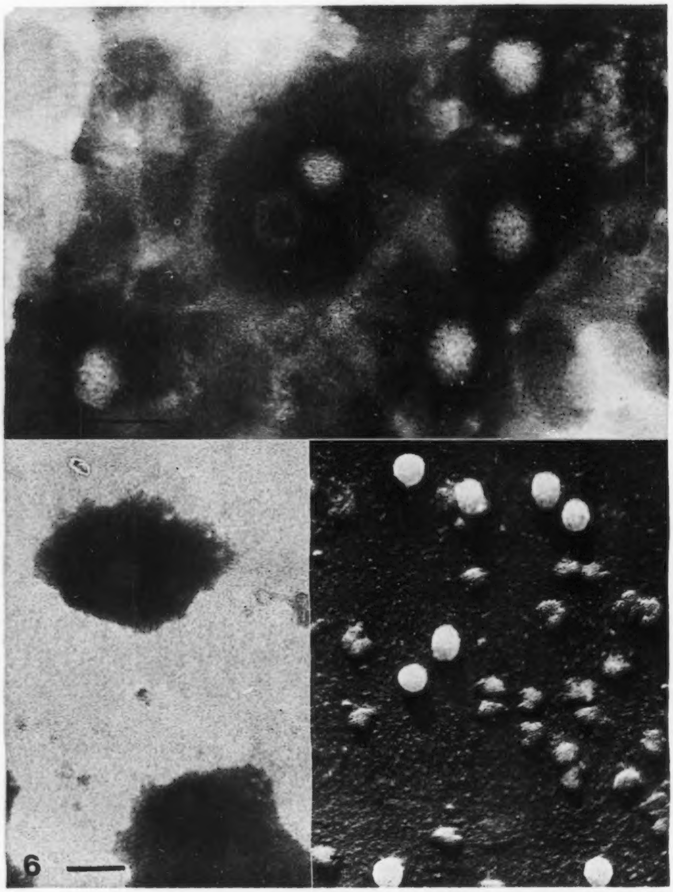

FIGURES 5–7.

FIG. *5. IPN virus particles from partially purified tissue culture extracts negatively stained with potassium phosphotungstate exhibiting icosahedral symmetry.* (The lines in Fig. 5–8 represent a measure of 100 nm.); FIG. 6. IPN virus particles purified in CsCl stained as in Fig. 5, measuring 70 nm in diameter; FIG. 7. IPN virus particles shadowed in palladium. A negative print is shown to illustrate the structure and features of the virus capsid.

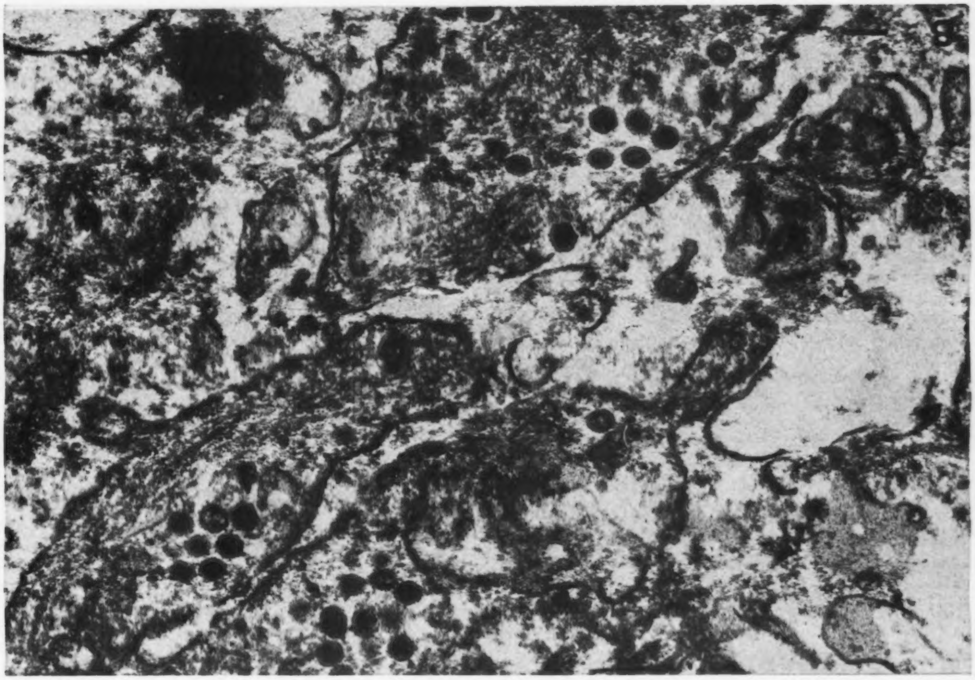

FIG. 8. Thin section of yearling brook trout (*Salvelinus fontinalis*) kidney naturally infected with IPN virus. Note the typical icosahedral virus particles. In many cases the virus particles appear to be localized in membrane bound regions.

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TABLE 1. Frequency of virus isolation from brood, 2-year, and yearling brook trout (*Salvelinus fontinalis*).

Date	No. sampled	No. with virus	
	Broodstock		
Apr. 24	11	11	
May 23	12	10	
June 6	16	13	
July 10	10	10	
Total	49	44	(91%)
	2-year-olds		
July 6	11	11	
July 10	10	9	
July 31	11	6	
Total	32	26	(81%)
	Yearlings		
July 3	11	0	
July 10	7	7	
July 14	8	0[a]	
July 13	7	1	
Aug. 30	7	5	
Sept. 5	49	41	
Total	89	54	(61%)

[a] Animals in this lot examined and found negative on July 14 were reexamined on September 5 and found to have a high incidence of virus infection.

TABLE 2. Relative concentrations of virus along the length of the kidneys and spleen of brood brook trout.

Animal number	Portion of kidneys[a]				Spleen
	Anterior	Midanterior	Midposterior	Posterior	
1	−	−	±	+	−
2	±	++	++	++	±
3	+	+	+	++	ND
4	−	−	−	±	±
5	+	++	++	++	+
6	−	±	−	±	−
7	+	+	++	++	±
8	−	±	−	++	−
Totals 8	4	6	5	8	5

[a] ++ indicates rapid CPE; + indicates moderate CPE; ± indicates slow CPE and/or CPE in only one of two culture tubes; − indicates no CPE; ND not done.

not identical to the one used to prepare the antiserum. Electron microscope examination of negatively stained virus particles from several isolates showed typical IPN virus particles (Fig. 5) measuring 70 nm in diameter compared to standard latex particles (Fig. 6). Examination of shadowed virus and latex particles also showed virus of similar dimensions (Fig. 7). Thin sections of both RTG-2 and CHSE-214 infected cells showed IPN virus in the cytoplasm. In addition a number of kidneys from naturally infected 2-year-old brook trout were found by electron microscopy to contain typical IPN virus particles (Fig. 8).

When sections of infected kidney cells were stained with hematoxylin–eosin, little histologic change was detected relative to normal uninfected cells.

ISOLATION OF VIRUS FROM BROOK TROUT

Virus was present in all three age-groups although some lots of yearlings (based on relatively small numbers) appeared to be free of infection (Table 1). Ninety-one percent of the brood brook trout and eighty-one percent of the 2-year-old fish were found to contain virus.

Among yearling brook trout the incidence of isolations was more variable. Two lots of fish examined in July yielded no virus. However, the same lots of fish from the same raceways were examined again on September 5 and found to have a high incidence of virus infection (41 of 49 examined or an isolation rate of 84%). Samples taken on August 30 were also from the same raceway and five of seven fish examined yielded virus. Because of this conversion from negative to positive it can be assumed that between the middle of July and end of August the yearling brook trout had undergone a massive IPN virus infection. Since there were no mortalities among the yearling brook trout during this period, evidently infection did not markedly affect these fish. The yearlings when received from the hatchery were in apparent good condition and showed no ill effects due to the virus. However, some of the fish included in the samples taken on September 5 had bacterial kidney disease and did show illness and mortalities.

ISOLATION OF VIRUS FROM VARIOUS ORGANS OF BROOD STOCK, 2-YEAR AND YEARLING BROOK TROUT

Two different lots of brood brook trout totalling 28 were examined. A total of 24 or 86% revealed the presence of virus. Of these 24 animals, virus was recovered from the kidneys of all of them, but the spleen yielded 16 or 54%, the liver 6 or 26%, testes none, and the ovary 2 or 7%. Virus isolation from feces was not determined since previous analyses showed this lot of animals to have little virus present in the feces.

Similar studies were made on the organs of 2-year-old brook trout. An identical pattern of isolation from the organs was obtained with about 80% positives from the kidneys, 70% positives from the spleen, and much lower isolations from the other organs tested.

Because yearling brook trout would most likely be of more recent infection than brood or 2-year-old animals, the rates of virus isolations from various organs were compared. Several lots of infected yearling brook trout were examined and the caeca were also added for analysis of pancreatic tissue. Of a total of 18 animals examined, 17 or 94% were positive for virus. Of these, 13 isolations (72%) were made from the kidneys, 10 (56%) from the spleen, 11 (61%) from the feces, 13 (72%) from the caeca, and 6 (33%) from the gonads. Compared to the older animals these yearlings exhibited different rates of virus isolation from the various organs. Although the caeca of older fish were not analyzed for virus in these animals, other studies showed that the caeca of older carrier fish rarely yield IPN virus.

In the previous analysis it was demonstrated that the kidneys were the one single pair of organs that more frequently yielded virus compared to other organs. Therefore it was decided to determine whether or not the entire kidney or certain portions of it were carrying the virus. At the same time, the spleen was also analyzed for virus content since it was also found to be frequently positive for virus. Table 2 gives the results from eight brood stock brook trout ($3\frac{1}{2}$-year-old) kidneys and spleens that were examined as described.

Of the eight animals examined only four yielded virus from all four portions of the kidneys. In these four animals the virus was also isolated from three of the spleens tested. The posterior portion of the kidneys appeared to contain as much virus and often more than other parts of the kidneys. The anterior portion contained the least amount of virus as well as yielding the least number of virus isolations. In addition, three of the eight carriers of virus did not yield virus from the spleen. Because of variable isolations from the different areas of the kidneys, it is possible to miss detection of a proportion of virus carriers. With these limited tests, apparently the posterior portion of the kidneys not only has higher levels of virus but also yields more virus than the other portions.

Similar experiments were done with several of the 2-year-old brook trout and they were also found to have more virus and more positive isolations from the posterior portion of the kidneys.

RELATIVE NUMBERS OF VIRUS IN ORGANS OF YEARLING BROOK TROUT

As shown earlier the yearling brook trout were apparently undergoing a recent active infection and the virus was present in most of the organs. Therefore, a few animals were examined to determine the relative virus content of certain organs. The organ extracts were tested for rapidity of CPE production as an indication of the amount of virus present. The results from eight animals are shown in Table 3.

The results presented a different spectrum of isolations and relative virus contents compared to those obtained with the older animals. Virus isolations were almost the same (Table 3) from all organs of infected yearling fish. This pattern of isolation contrasts with that of the 2-year-olds and brood stock, and indicates that in recently infected animals there is a general distribution of virus throughout the body.

ISOLATION OF VIRUS FROM RAINBOW TROUT

Virus isolation studies in two strains (Tasmanian and Soap Lake) of rainbow trout showed that the incidence of virus carriers in rainbow trout was considerably less than that of brook trout (Table 4). The Tasmanian strain showed a carrier incidence of 14%, whereas the Soap Lake strain showed an incidence of 17%.

Of the 58 rainbow trout examined (2½ years old), only one yielded virus. Similar results were obtained for the yearling rainbow trout where only one isolation was made out of a total of 46 animals examined.

TABLE 3. Virus in organs of yearling brook trout.

Specimen no.	Organ[a] Kidney	Spleen	Caecum	Feces	Gonads
1	+	±	±	±	±
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	±	–	±	–
6	+	±	+	+	–
7	–	–	–	±	–
8	+	+	+	+	–
Total 8	7	7	6	8	4

[a] + indicates rapid CPE; ± indicates slow CPE and/or CPE in only one of two culture tubes; – indicates no CPE observed.

Because of the low incidence of virus carriers among the rainbow trout much less information was obtained with respect to the organs containing virus. Of those animals shown in Table 4, none of the feces examined from April 24 to July 21 showed the presence of virus. Therefore, only the kidneys, spleen, and gonads were examined. Only the positive isolations of 48 rainbow trout examined on August 8 are shown in Table 5. Of the 10 animals that yielded virus there was a total of 12 isolations from the kidneys, spleen, and gonads numbering eight, three, and one, respectively, for each of the organs. These rather limited results indicate that the kidney is one the single organ that is useful for the detection of carriers in a population of rainbow trout where the carrier rate is small.

TABLE 4. Virus isolations from rainbow trout (*Salmo gairdneri*).

Date	No. with virus/No. sampled	
Brood stock — age 3½ yr (Tasmanian strain)		
Apr. 24	0/12	
July 6	1/6	
July 14	1/7	
July 31	0/10	
Aug. 8	6/24	
Total	8/59	(14%)
Brood stock — age 3½ yr (Soap strain)		
July 6	2/10	
July 10	3/10	
July 31	0/10	
Aug. 8	4/24	
Total	9/54	(17%)
2½ year old (strain not known)		
July 14	0/5	
July 14	0/5	
July 31	0/13	
July 31	0/27	
Aug. 8	1/7	
Total	1/58	(2%)
1½ year old (strain not known)		
May 29	0/12	
July 31	0/27	
Aug. 30	1/7	
Total	1/46	(2%)

TABLE 5. Isolation of virus from brood rainbow trout.

No.[a]	Kidneys	Spleen	Gonads
1	ND	–	+
2	+	±	–
3	±	–	–
4	±	–	–
5	–	±	–
6	±	–	–
7	±	±	–
8	±	–	–
9	±	–	–
10	±	–	–
Total	8	3	1

[a] + indicates good development of CPE in duplicate tube cultures; ± indicates slow development of CPE and/or CPE in only one tube; – no CPE detected.

The slow development of CPE in the culture tubes and the frequent necessity of repassage demonstrated that the numbers of virus in these carriers are small, approaching the limits of detection by normal diagnostic tube-culture methods. Undoubtedly a higher isolation rate could be achieved by more vigorous isolation methods.

ABSENCE OF IPN VIRUS IN FRY DURING THE SUMMER OF 1972

There was an increase in mortality in young brook and rainbow fry at the hatchery beginning in May and increasing into June. The cause of this mortality was diagnosed as bacterial gill disease. At first, however, it was suspected that IPN might also be present. Numerous attempts to isolate the virus from young of both species did not reveal a single one to be infected. The reason for this lack of transmission of IPN infection to the young was due to rigid sanitary precautions that were instituted at the hatchery during the period of this study.

Discussion

The virus isolated from brook and rainbow trout at the Maligne River Hatchery in RTG-2 and CHSE-214 cells was identified to be IPN virus by (a) its morphology, (b) resistance to ether, and (c) by partial neutralization by an antiserum prepared against a known strain of IPN virus obtained from another laboratory. During the course of investigation it was found that cell line CHSE-214 was equally effective compared to RTG-2 cells for isolation purposes. Since the CHSE-214 cell line when infected with IPN virus shows a different type of cytopathology, namely one of rounding of the cells, compared to that of other cell lines, the type of CPE is a characteristic feature of the cell line and not of the virus.

The morphology of the virus, both in shadowed and in negatively stained preparations, showed the virus to be typically icosahedral in shape measuring 70 nm in diameter. This latter result agrees reasonably well with the recent studies by Moss and Gravell (1968), Lightner and Post (1969), Cohen and Scherrer (1972), and Kelly and Loh (1972) who reported dimensions of 65, 57, 63, and 74 nm, respectively. A feature characteristic of IPN virus multiplication in cells appears to be aggregation of the virus in clumps, often within membrane structures. This characteristic was found in tissues of naturally infected animals (Fig. 8), as well as in tissue culture cells. This feature was also observed by Lightner and Post (1969) in pancreatic acinar tissue of rainbow trout, by Moss and Gravell (1968) in FHM tissue culture cells, and by Wolf and Quimby (1971) in RTG-2 tissue culture cells. Relatively large numbers of virus can be found in kidney tissue even in the absence of any clinical disease.

The high rate of isolations of virus from hatchery brook trout indicated that all the older animals were most likely infected at some time in the past and that the majority have remained carriers of the virus for a considerable time. Similar reports for high virus isolation rates for brook trout have been found by Billi and Wolf (1969). This continued presence of IPN virus carriers would make it very difficult to eradicate the virus from a particular watershed once the virus was introduced.

In the case of yearling brook trout it is interesting to speculate on the time when the different lots were infected. In one lot it can be stated that the yearling brook trout were undergoing infection during the period of the study and not infected the previous year as young fry (Table 1). Another factor that tends to substantiate that active infections were occurring in the absence of symptoms is the large numbers of virions present throughout the various organs (Table 3). This is the case when yearling brook trout are artificially infected in the laboratory (unreported experiments).

Previous workers have emphasized the analysis of feces and sex products for virus to detect carriers of IPN (Wolf et al. 1963; Billi and Wolf 1969). Evidently the organs of carriers may in fact harbor many virions when the feces or sex products rarely contain them. The results obtained with brook and rainbow trout present evidence that kidneys are an important organ to examine for the detection of animals carrying the virus. This importance of the kidneys for virus isolation appeared to be true for all ages of fish. In the yearling fish, virus could be readily isolated from other organs as well. With the older brood trout, next to the kidney the spleen appeared to contain virus most frequently. In larger animals where only a small portion of the kidneys can be effectively sampled, it was shown that the posterior part of the kidneys was more likely to contain virus. However, because of this regional localization of the virions, it might be wise to obtain samples from several different parts of the kidneys for virus isolation.

The rate of virus isolation from rainbow trout was much less than that obtained for brook trout indicating that the carrier rate among rainbow trout is very low. Most epidemiological studies of IPN infections have been with brook trout and it may be that differing carrier rates occur in different salmonids.

With regard to the relationship of IPN infections and different species it is interesting to speculate on the susceptibility of older rainbow trout to IPN virus infection. The layout of the hatchery was such that the water from the raceways all drained into a large pond in which both strains of rainbow brood stock were kept. Because the yearling brook trout that were kept in the raceways were all infected, and virus could be readily detected in their feces, obviously large numbers of virions were being fed into the brood pond. The very low rate of virus isolation from rainbow trout would indicate a general resistance to virus infection that could be due either to a natural resistance of these older rainbow trout, or to an actively acquired immunity due to infection at an earlier age. Nevertheless as in the case of the brook trout, the kidneys were found to be the organ most likely to contain the virus and not the sex organs, feces, or caeca.

The lack of isolation of IPN virus from fry of both brook and rainbow trout during the study is interesting and shows that it is possible to set virus-free eggs from carrier brood stock if proper sanitary measures are taken. Similar reports of raising IPN virus-free trout from diseased stock has been reported (Wolf et al. 1968). However, the rearing of any salmonid with a water source containing carriers is not recommended since the virus will most likely be introduced in time by contaminated water.

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Lipids and Fatty Acids of the American (*Crassostrea virginica*) and European Flat (*Ostrea edulis*) Oysters from a Common Habitat, and After One Feeding with *Dicrateria inornata* or *Isochrysis galbana*

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The oysters *Crassostrea virginica* and *Ostrea edulis* from a common habitat showed species-oriented fatty acid composition patterns for total lipids and lipid fractions. Attempts to modify these patterns by offering the unicellular algae *Dicrateria inornata* and *Isochrysis galbana* as food suggest that the oysters rapidly convert qualitatively or quantitatively unusual fatty acids to these species-oriented compositions, although only in the combination of *D. inornata* and *O. edulis* was enough fatty acid taken up to modify the oyster fatty acid composition in an easily detectable way. The unusual C_{22} nonmethylene-interrupted diunsaturated fatty acid components were not found in the *O. edulis* used for this study.

Watanabe, T., and R. G. Ackman. 1974. Lipids and fatty acids of the American (*Crassostrea virginica*) and European flat (*Ostrea edulis*) oysters from a common habitat, and after one feeding with *Dicrateria inornata* or *Isochrysis galbana*. J. Fish. Res. Board Can. 31: 403–409.

La composition en acides gras des huîtres *Crassostrea virginica* et *Ostrea edulis* provenant d'un même habitat exhibe des différences spécifiques dans les lipides totaux et les fractions de lipides. Des essais effectuées en vue de modifier ces caractéristiques, en nourrissant les huîtres des algues unicellulaires *Dicrateria inornata* et *Isochrysis galbana*, suggèrent que les huîtres transforment rapidement les acides gras qualitativement et quantitativement anormales pour obtenir ces compositions propres à l'espèce. Par ailleurs, seule la combinaison *D. inornata* et *O. edulis* permet une absorption suffisante d'acide gras par l'huître pour modifier sa composition d'une façon facilement décelable. Chez les *O. edulis* utilisés pour cette étude, nous n'avons pas trouvé les composants peu communs d'acides gras en C_{22} diinsaturés non interrompus par le méthylène.

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The importation of the European flat oyster (*Ostrea edulis*) to Prince Edward Island for research purposes (R. Drinnan, personal communication) afforded a unique opportunity to compare its lipid and fatty acid composition with those of the native American oyster (*Crassostrea virginica*).

The nutritional requirements of oysters and other molluscs are poorly understood in many respects. Practical aspects of oyster propagation and culture have led to several studies on the nutritional requirements of young oysters (Galtsoff 1964; De-Mort 1970; Walne 1970). Other studies on oyster lipids and fatty acids have usually involved comparisons with other molluscs, or in varying detail appropriate seasonal variations in composition (Calzolari et al. 1971; Cerma et al. 1970; Stancher 1971). Some rudimentary preliminary studies with *Skeletonema costatum* at the Halifax Laboratory indicated that oysters could ingest substantial quantities of unicellular algae (R. G. Ackman, unpublished data). Accordingly lipid and fatty acid composition changes arising from the diet might be detected on a gross scale not requiring radioactive tracers. We have therefore examined the

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effects of ingestion of two species of unicellular algae, *Dicrateria inornata* and *Isochrysis galbana* on the two species of oyster, *C. virginica* and *O. edulis*. The results were essentially negative as the oyster fatty acid compositions were species-oriented, rather than diet-oriented, in both control and experimental animals. A complete and detailed publication of fatty acid compositional detail has appeared elsewhere (Watanabe and Ackman 1972), and this report is limited to the fatty acids of significance from a quantitative or biochemical point of view.

Experimental

The axenic cultures of *D. inornata* and *I. galbana* were grown at 20–25 C in sunlight, in an enriched seawater medium, at Ellerslie, Prince Edward Island. *Crassostrea virginica* of local stocks, and *O. edulis* of British origin that had been grown for 1 yr in Ellerslie, were taken from stock on the same tray that had been suspended in an estuary near the Ellerslie laboratory for several months prior to experimental feeding. Ten oysters were placed in a polyethylene tray (45 × 30 × 15 cm) with 4 liters of culture. The cell density of the culture was determined every 30 min by the packed volume method. The culture was replaced when about half of the cells had been filtered out. At the conclusion of feeding (approximately 3 h of active ingestion out of a total exposure of 6 h) the oysters were placed in plastic bags and cooled to temperatures near ice water during transport to the Halifax Laboratory. Subsequently storage was at 3 C until used. An equal number of control oysters of each species that had not been fed were also cooled and taken to Halifax under the same conditions.

Cultures were harvested by gentle centrifugation and the pellets of algal cells drained to determine approximate wet weights (Table 1). Subsequent operations were carried out under an atmosphere of nitrogen. Methanol and/or chloroform were used to transfer the cells to separatory funnels with water in limited amounts to maintain a monophasic solvent for 15–30 min. Additional water was then added to arrive at the final ratios of solvents indicated by Bligh and Dyer (1959). The bottom layer was filtered and stripped for lipid recovery (Table 1).

Extractions of oysters were carried out on total wet organic tissues with chloroform–methanol (Bligh and Dyer 1959). Polar lipids were separated on and recovered from a styrene divinylbenzene copolymer bead gel column (Sipos and Ackman 1968). Nonpolar lipids were then separated into sterol esters, glyceryl ethers, triglycerides, free fatty acids (in the case of *O. edulis*), and sterols on silica gel thin-layer chromatography plates. Details for lipids other than phospholipids and triglycerides may be found in Watanabe and Ackman (1972). Triglycerides were transesterified directly with 7% BF_3-MeOH. All other lipid fractions were saponified and nonsaponifiables were extracted and weighed. The recovered fatty acids were esterified with BF_3-MeOH reagent. Removal of nonsaponifiable materials from lipids, preparation of methyl esters (by BF_3-MeOH esterifications of acids, or by transesterification of triglyceride), and all gas–liquid chromatography operations (wall-coated open-tubular columns coated with butanediolsuccinate polyester), followed procedures described previously (Sipos and Ackman 1968; Ackman and Hooper 1970). Particular care was taken to avoid confusion between branched-chain acids and plasmalogen artifacts (Ackman 1972) as oysters are rich in these lipids (Sampugna et al. 1972).

Results and Discussion

Table 1 and 2 indicate that in both control and experimental oysters, there are differences in composition between *C. virginica* and *O. edulis*. For example in fatty acids from all lipids, our multiple analyses show that the latter species is two to three times higher in 18:0, whereas the converse is true for 18:1. The successor 20:1[1] acids, produced from 18:1 acids by chain extension (discussed by Ackman and Hooper 1973), are also about twice as high in *C. virginica*. In the polyunsaturated acids it seems that *C. virginica* accumulates 20:5ω3 and 22:6ω3 in about equal proportions but *O. edulis* does not extend as much fatty acid to 22:6ω3, so that the ratio for these two acids is about 2:1.

A highly specific, species-oriented difference in oyster fatty acids can be observed in the data for 15:0 and 17:0 (Table 2). In *O. edulis* these two acids comprised as much as 10% of total lipid as against a third of this value in *C. virginica*. This effect is associated with the dominant polar lipids, and with the free fatty acids isolated from *O. edulis* but not from *C. virginica* (Watanabe and Ackman 1972). Triglyceride percentages for these acids are about the same in both species. Total saturated acids are higher in the former (e.g. 77% in control total) than in the latter (e.g. 41% in control total), but not enough to account for these unusual figures for the two odd-numbered fatty acids. This result for *O. edulis* is foreign to our detailed studies on the saturated fatty acids of molluscs (Ackman et al. 1971) and to the analytical results of others cited in this paper.

Our results, including the results of feeding discussed below, indicate that *C. virginica* and *O. edulis* deposit fatty acids in slightly different and species-oriented compositions. The conclusion that an animal-specific composition is a consideration overriding algal fatty acid compositions has also

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[1] Shorthand notation for chain length: number of double bonds and where applicable positions of penultimate bonds counted from terminal methyl group.

TABLE 1. Comparison of lipids isolated from dietary *Isochrysis galdana* and *Dicrateria inornata* with those from *Crassostrea virginica* and *Ostrea edulis*.

			C. virginica			*O. edulis*		
	I. galbana	*D. inornata*	Control	Experimental fed *Isochrysis*	Experimental fed *Dicrateria*	Control	Experimental fed *Isochrysis*	Experimental fed *Dicrateria*
Number of animals	–	–	9	10	10	15	10	10
Average weight of whole body (*g*) (including shell)	–	–	53.0	56.9	58.0	22.6	25.1	26.0
Drained weight of sample (*g*) (meat)	31.2	25.0	45.9	45.4	43.2	25.0	23.4	21.0
Algae taken up (*g*)	–	–	–	2.2	5.0	–	2.7	6.3
Calc. lipid taken up (*mg*)	–	–	–	11	87	–	14	109
Total lipid recovered (*mg*)	160	432	734	663	826	415	408	321
Total lipid recovered (%)	0.5	1.7	1.6	1.5	1.8	1.7	1.7	1.5
Fatty acids recovered (% of total lipids)	31.3	24.4	60.8	62.3	64.6	41.8	37.8	39.1
Polar lipid (% recovered from lipid)	41.1	21.7	45.7	58.3	45.3	53.8	47.6	55.0
Fatty acids recovered (% of polar lipids)	51.5	42.3	67.0	53.9	59.8	26.1	29.7	29.1
Nonpolar lipid (% recovered from lipid)[a]	53.4	66.5	53.6	41.1	50.5	40.9	47.3	37.2
Fatty acids (% of nonpolar lipid)	4.7	15.1	–	–	–	–	–	–
Triglyceride (% of nonpolar lipid)	–	–	37.1	21.1	30.1	58.3	33.0	37.0
Free fatty acids (% of nonpolar lipid)	–	–	trace	trace	trace	9.6	4.1	11.7
Sterol (% of nonpolar lipid)	–	–	17.5	32.1	21.2	28.4	24.5	37.0

[a] Steryl esters and glyceryl ethers were, respectively, 3–5% in *C. virginica*, 6–18% in *O. edulis*.

been reached for the brine shrimp *Artemia salina* raised on several different algal species (Hinchcliffe and Riley 1972). Our two control fatty acid compositions were evidently achieved by oysters from the wide range of normal food cells and particles available in the tray location in Ellerslie. Our feeding experiment results do not indicate the nutritional effectiveness of our algae. A food supplement of the unicellular alga *Tetraselmis suecica* has shown improved spawning, apparently associated with lipids (Helm et al. 1973).

The open tubular gas–liquid chromatography assures that in our analyses there was no overlap of important fatty acids with other components. However, a few of our analyses are suspect as partial autoxidation of polyunsaturated acids may have occurred. The majority for *C. virginica* show the 20:5ω3 total as 20% for total and polar lipids or about the same as reported for three marine pelecypods by Gardner and Riley (1972). In contrast, Bannatyne and Thomas (1969) show 35% for total lipids of *Ostrea lutaria* (New Zealand), and correspondingly less 16:0 (18.5%). Pacific oysters (*Crassostrea gigas*) and other shellfish also seem to be high in these acids (Gruger 1967). Presumably our lower value is another oyster

TABLE 2. Comparison of some of the fatty acids in lipids of the algae *I. galbana* and *D. inornata* and in the lipids of the oysters *C. virginica* and *O. edulis* with and without feeding on the algae.

Algae and fatty acid				*C. virginica* lipids						*O. edulis* lipids					
	Algae lipids			Total		Polar		TG		Total		Polar		TG	
	Total	Polar	Nonpolar	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
I. galbana															
14:0	10.6	15.0	0.4	3.5	2.8	2.0	1.6	4.4	4.2	8.9	8.4	6.0	7.0	8.1	5.9
15:0	1.3	1.4	0.6	1.3	1.6	1.6	1.2	1.4	1.7	3.8	3.6	3.5	3.1	2.1	1.7
16:0	22.2	23.0	6.9	28.9	29.9	26.7	23.9	34.8	38.6	33.8	37.8	31.5	25.1	42.0	38.0
17:0	1.1	0.4	1.7	1.6	1.9	1.9	1.6	2.0	2.3	3.0	3.6	2.5	2.9	3.1	2.7
18:0	2.3	1.7	12.1	3.6	4.5	6.2	6.3	2.2	3.7	10.1	10.9	12.7	12.2	7.5	7.2
16:1ω9	1.4	0.3	TRA	1.0	0.9	0.6	0.5	1.3	1.6	1.7	2.0	1.5	4.4	1.7	1.2
16:1ω7	14.3	12.4	1.5	3.2	2.1	1.8	1.5	4.0	4.3	4.3	4.1	2.5	2.3	5.9	6.2
18:1ω9	3.8	4.7	28.7	3.9	3.8	3.7	4.7	4.8	4.6	4.1	3.5	2.7	4.6	5.4	4.9
18:1ω7	9.3	11.7		4.3	3.4	3.1	3.8	5.6	4.8	2.7	3.2	1.3	0.8	5.9	6.4
20:1ω11	0.3	0.3	1.2	1.5	1.7	1.9	2.0	0.8	0.9	0.5	0.8	1.0	1.2	0.6	0.5
20:1ω9	TRA	TRA	3.7	0.6	1.1	1.5	1.2	0.5	0.7	0.9	0.4	0.8	0.4	0.4	0.3
20:1ω7	0.9	0.2	2.1	2.9	3.9	4.9	4.6	1.5	1.8	1.9	1.9	2.2	1.7	2.6	2.7
22:1ω13 + 11	TRA	0.1	4.9	NSA	NSA	NSA	NSA	NSA	NSA	0.3	NSA	NSA	NSA	0.2	NSA
22:1ω9	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA	NSA	0.2	NSA	0.1	0.1	0.1	0.1
22:1ω7	NSA	NSA	NSA	0.3	0.4	0.8	1.8	TRA	0.2	0.5	TRA	1.3	1.2	0.6	0.4
16:3ω3	0.4	1.0	NSA	0.1	0.2	0.7	0.6	TRA	TRA	NSA	NSA	NSA	NSA	NSA	NSA
18:2ω6	2.3	2.3	1.7	2.0	1.8	0.9	1.1	2.6	2.8	0.8	2.1	0.9	2.9	1.3	1.9
18:3ω6	0.2	0.1	0.7	TRA	TRA	NSA	NSA	0.1	0.1	0.3	0.1	NSA	TRA	0.1	0.1
18:3ω3	0.4	3.0	0.6	3.3	2.9	3.8	3.3	2.9	2.7	3.8	2.4	4.9	4.2	1.1	1.8
18:4ω3	8.0	2.8	12.1	2.6	2.0	1.1	1.2	3.0	2.8	1.0	0.8	1.0	1.0	0.6	1.2
20:4ω6	0.1	0.5	1.3	2.3	2.8	4.6	4.7	1.1	0.7	0.7	0.6	2.6	1.9	0.5	0.8
20:5ω3	7.2	8.7	5.8	11.2	8.4	8.1	9.8	9.1	6.1	3.4	5.0	6.9	6.6	2.2	6.2
22:6ω3	4.3	4.8	NSA	9.7	9.4	10.3	11.8	4.7	4.9	1.3	2.4	2.3	1.7	0.9	2.8
D. inornata															
14:0	11.9	12.0	10.5	3.5	2.6	2.0	1.8	4.4	4.7	8.9	10.2	6.0	7.1	8.1	8.0
15:0	3.7	2.7	4.4	1.3	1.2	1.6	1.5	1.4	1.7	3.8	3.7	3.4	3.8	2.1	1.9
16:0	30.3	31.7	24.4	28.9	24.8	26.7	23.0	34.8	42.1	33.8	33.5	31.5	27.8	38.0	40.5
17:0	0.6	1.3	0.8	1.5	1.5	1.9	2.0	2.0	2.3	3.0	2.8	2.5	2.0	3.1	2.6
18:0	2.4	5.0	3.9	3.6	6.2	6.2	5.2	2.2	2.6	10.1	12.7	12.7	12.9	7.2	7.6
16:1ω9	1.5	1.6	11.4	1.0	0.8	0.6	0.4	1.3	1.3	1.7	2.0	1.5	4.7	1.7	1.8
16:1ω7	10.2	9.7		3.2	2.0	1.8	1.0	4.0	3.3	4.3	6.1	2.5	4.7	5.9	6.1
18:1ω9	10.1	11.8	10.5	3.9	3.9	3.7	3.0	4.8	5.2	4.1	4.4	2.7	5.7	5.4	4.8
18:1ω7	4.5	4.8	4.1	4.3	3.4	3.1	2.4	5.6	4.6	2.7	2.0	1.3	1.2	5.9	7.4
20:1ω11	NSA	NSA	NSA	1.5	1.4	1.9	2.4	0.8	0.8	0.5	0.3	1.0	0.8	0.6	0.5
20:1ω9	3.5	3.9	3.6	0.6	0.8	1.5	1.6	0.5	0.6	0.9	0.3	0.8	0.8	0.4	0.4
20:1ω7	0.4	0.6	0.7	2.9	3.0	4.9	5.4	1.5	1.4	1.9	0.9	2.2	2.0	2.6	2.3
22:1ω13 + 11	1.2	1.1	1.5	NSA	NSA	NSA	NSA	NSA	NSA	0.3	0.8	NSA	NSA	0.2	0.1
22:1ω9	0.5	0.7	0.3	NSA	NSA	NSA	NSA	NSA	NSA	0.2		0.1	0.1	0.1	0.1
22:1ω7	0.1	0.2	0.1	0.3	0.4	0.8	0.8	TRA	0.1	0.5		1.3	2.0	0.6	0.7
16:3ω3	5.8	0.9	11.7	0.1	0.2	0.7	0.6	TRA	TRA	NSA	NSA	NSA	NSA	NSA	NSA
18:2ω6	2.5	1.3	0.8	2.0	1.9	0.9	1.1	2.6	2.5	0.8	1.9	0.9	3.9	1.3	1.3
18:3ω6	TRA	0.1	NSA	TRA	TRA	NSA	TRA	0.1	0.1	0.3	0.3	NSA	0.1	0.1	TRA
18:3ω3	3.1	1.3	2.5	3.3	3.0	3.8	5.2	2.9	2.6	3.8	1.6	4.9	2.8	1.1	1.1
18:4ω3	0.3	0.2	0.2	2.6	2.8	1.1	1.0	3.0	3.4	1.0	0.3	1.0	0.9	0.6	0.5
20:4ω6	0.2	0.3	0.2	2.3	3.0	4.6	4.8	1.1	0.7	0.7	TRA	2.6	1.4	0.5	0.5
20:5ω3	0.5	0.1	0.2	11.2	12.5	8.1	9.9	9.1	5.9	3.4	3.5	6.9	6.5	2.2	2.2
22:6ω3	TRA	0.1	NSA	9.7	15.2	10.3	10.6	4.7	2.8	1.3	2.7	2.3	3.8	0.9	0.8

species peculiarity. For both species of oysters from near Ellerslie, the very warm water in surface layers (25–28 C) needs to be recorded as a factor as many samples collected elsewhere may have come from colder and deeper waters. Seasonal studies on lipids of several molluscs (Stancher et al. 1971) show in most species more lipid in midsummer than midwinter, and correspondingly 20:5ω3 and 22:6ω3 are only half the percentage in midsummer that they are in midwinter (Cazolari et al. 1971).

The two algae were selected to have different classes of unsaturated fatty acids of possible nutritional interest in oysters. Thus *D. inornata* lacked longer-chain (C_{20} and C_{22}) polyunsaturated fatty acids. The 16:3ω3 and 18:3ω3 acids were, however, potential sources of the longer-chain acids in animals if converted by chain extension and dehydrogenation. The 18:1 and 20:1 acids were similar in isomer ratio to animal lipids with 18:1ω9>18:1ω7. *Isochrysis galbana* was known to contain 18:4ω3 and 20:5ω3 and also differed (Table 2) in isomers, with 18:1ω7>18:1ω9. Our fatty acid composition for *I. galbana* (Table 2) is unusual in the high percentage of 14:0, but appears to contain proportions of fatty acids intermediate to those reported in two different studies, that of DeMort (1970) having 10.6% 14:0, 0.5% 18:4ω3, and 14.4% 20:5ω3, and that reported by Chuecas and Riley (1969) having only 0.6% 14:0, 17.2% 18:4ω3, and 3.1% 20:5ω3. Possibly this alga is unusually sensitive to culture conditions. *Isochrysis galbana* is one of the algae most suitable for promoting growth in small (larval) *O. edulis*, or in the native Pacific oyster of Oregon, *Ostrea lurida* (DeMort 1970) and may be a generally satisfactory bivalve food (Walne 1970, 1972). The two oyster species in our experiment have high specific filtration rates for *I. galbana* (Walne 1972).

The known uptake of algae by the oysters and the analyses of the algal lipids (Table 1) indicated that the maximum possible uptake of algal fatty acids would be a reasonable proportion of total oyster fatty acids only in one combination. *Crassostrea virginica* took up algae which would yield 0.8% of *I. galbana* fatty acids in recovered oyster fatty acid, or 4% in the case of *D. inornata*. *Ostrea edulis* consumed about the same weight of algae for only half the oyster meat weight. Also in *O. edulis* total recovered fatty acids were only about half as much as in *C. virginica*. Therefore *I. galbana* fatty acid potentially accounted for 2.8% of total fatty acids recovered and *D. inornata* for 21%. Examination of the fatty acid data in Table 2 shows that even in the latter favorable case the only striking experimental result is the absence of 16:3ω3 in *O. edulis*. The maximum possible deposition of unchanged 16:3ω3 would have given an *O. edulis* fatty acid composition containing 1.2% of this acid. In *C. virginica* controls and experimental animals fed *D. inornata* the corresponding composition would have included deposits of not more than 0.2% of 16:3ω3, or approximately the level observed in total lipids of both control and experimental animals. Animals fed *D. inornata* show the same basic proportions for this acid. Its absence in *O. edulis* suggests that fatty acids are very rapidly assimilated and either catabolyzed or transformed into the common higher homologues such as 20:5ω3. Moreno et al. (1970) have shown that the mollusc *Mesodesma mactroides* converts 50% of labelled 18:2ω6 into higher dienoic acids in 6 h. The absence of various C_{16} polyunsaturated acids, which are commonly found in phytoplankters, in grazing and filter-feeding molluscs is notable (Calzolari et al. 1971; Stancher et al. 1971; Gardner and Riley 1972) and probably extends to many other marine invertebrates (Culkin and Morris 1970).

Not much is known about storage of intact cells or lipids in the digestive diverticula, although lipids are held for several days under normal conditions in the scallop *Chlamys hericia* (Vassalo 1973). Feeding bivalves with algae labelled with P^{32} also led to short-term accumulation of label in the digestive gland. However, both of these experiments differed somewhat from ours in that the animals were held in normal conditions after feeding. We observed copious pseudofeces production during the 3 h of active feeding, especially with *C. virginica*. The extra exposure time of 3 h was felt adequate to guarantee satiation with algae. On opening oysters after several days of storage at 1–3 C, a temperature in which they are dormant as well as in an anaerobic state, the green of chlorophyll was observed to be diffused throughout the body in the experimental animals. The control animals were the normal off-white color. The 16:3ω3 residues in *C. virginica* suggests that this need not represent intact cells but may include those subject to partial degradation during phagocytosis as discussed by Ackman and Hingley (1968).

While this work was in progress, it became apparent that the fatty acid patterns for monoethylenic acids from *C. virginica* and other molluscs differed significantly from those normally observed in marine oils and lipids (for examples of the latter see Ackman and Castell 1966 and Addison et al. 1972). A partial elaboration of structures indicates that the unusual "monoethylenic" components are eicosadienoic and docosadienoic acids, probably of 5, 13 and 5, 15 unsaturation for the (20:2) acids and 7, 15 and 7, 17 unsaturation for the (22:2) acids (Ackman

TABLE 3. Weight percentage of unusual nonmethylene-interrupted fatty acids from *C. virginica* total lipids and various fractions with and without feeding on *I. galbana* and *D. inornata.*

Fatty acid (NMID)	Control	Experimental fed *Isochrysis*	Experimental fed *Dicrateria*
		Total lipid	
(20:2)	2.31	1.84	2.02
(22:2)	2.48	4.01	2.83
		Polar lipid	
(20:2)	1.29	1.27	1.46
(22:2)	6.09	6.39	6.63
		Triglyceride	
(20:2)	2.42	2.23	2.11
(22:2)	0.56	0.81	0.56

and Hooper 1973). The ( ) indicates that these do not belong to the usual methylene-interrupted polyunsaturated families of fatty acids. The C_{18} unknowns have not been characterized at the time of writing. It is probable that the high proportions of 20:1 and 22:1 or 22:2 reported by some authors, using packed-column gas–liquid chromatography for the analysis of mollusc lipids, are in fact partly appropriate nonmethylene-interrupted diethylenic acids (e.g. Ackman et al. 1971; Jeffries 1972; Farrington et al. 1973).

Because of the novelty of these results we have isolated the data for the (20:2) and (22:2) acids of *C. virginica* in Table 3. There still remain some unidentified peaks in the C_{20} and C_{22} regions, but these apparently occur unsystematically. The (20:2) and (22:2) acids were earlier found in some *O. edulis* samples (Watanabe and Ackman 1972) but were not found in the *O. edulis* of the time of feeding with *I. galbana* and *D. inornata*. There is no obvious explanation for this.

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Feeding Activity and Rates of Digestion of Northern Squawfish (*Ptychocheilus oregonensis*)

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STEIGENBERGER, L. W., AND P. A. LARKIN. 1974. Feeding activity and rates of digestion of northern squawfish (*Ptychocheilus oregonensis*). J. Fish. Res. Board Can. 31: 411–420.

Trapnet catches of northern squawfish (*Ptychocheilus oregonensis*) at Griffen and Cultus lakes, British Columbia, indicated peak activity and feeding in the twilight and dark hours. At Griffen Lake, squawfish force-fed 2.0 g of redside shiner (*Richardsonius balteatus*), and held at 17–20 C, digested at a rate of roughly 14%/h. At Cultus Lake, squawfish trapped during the peak of feeding activity, and held at 10–12 C, were sacrificed at 6-h intervals, and showed a digestion rate of sockeye salmon (*Oncorhynchus nerka*) smolts of 14%/h. Larger fish ate larger volumes of food. Regression techniques are not completely adequate for describing the course of digestion, which apparently involves an initial period of retention of food, followed by a fairly rapid evacuation of most of the ingested material. Most fish cleared their stomach contents in 24 h, and one half had done so between 12 and 18 h. In the laboratory, squawfish force-fed rainbow trout (*Salmo gairdneri*) had rates of digestion which increased with temperature, from about 5%/h at 4–6 C, to 40–50%/h at 24 C. Larger weights of food were digested at slower rates, but there were no consistent differences in rate of digestion by different sizes of squawfish.

The rates of digestion are higher than those reported for various predatory European fishes.

STEIGENBERGER, L. W., AND P. A. LARKIN. 1974. Feeding activity and rates of digestion of northern squawfish (*Ptychocheilus oregonensis*). J. Fish. Res. Board Can. 31: 411–420.

Les prises de sauvagesses du nord (*Ptychocheilus oregonensis*) au filet-trappe dans les lacs Griffen et Cultus, Colombie-Britannique, indiquent des pics d'activité et d'alimentation aux heures de demi-jour et de noirceur. Au lac Griffen, des sauvagesses du nord gravées de 2.0 g de ménés roses (*Richardsonius balteatus*) et maintenues à 17–20 C ont un taux de digestion d'environ 14%/h. Au lac Cultus, des sauvagesses du nord capturées dans des trappes au moment du pic d'alimentation et maintenues à 10–12 C ont été tuées à intervalles de 6 h et ont digéré des smolts de saumon nerka (*Oncorhynchus nerka*) au taux de 14%/h. Les grands poissons absorbent un plus grand volume de nourriture que les petits. Les calculs de régression ne sont pas tout à fait adéquats pour décrire le cours de la digestion qui, semble-t-il, comporte une phase initiale de rétention des aliments, suivie d'une rapide évacuation de la plus grande partie du matériel ingéré. La plupart des poissons ont un transit intestinal de moins de 24 h, et la moitié entre 12 et 18 h. Au laboratoire, des sauvagesses du nord gavées de truites arc-en-ciel (*Salmo gairdneri*) ont un taux de digestion qui passe d'environ 5%/h à 4–6 C à 40–50%/h à 24 C. La digestion est plus lente lorsque le poids ingéré est plus grand, mais il n'y a pas de différence uniforme dans le taux de digestion de sauvagesses du nord de différentes tailles.

Les taux de digestion sont plus élevés que ceux qui ont été signalés chez divers poissons prédateurs d'Europe.

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THE squawfish (*Ptychocheilus oregonensis*) is well known as a predator of juvenile salmon and other fishes in Pacific drainages (Clemens and Munro 1934; Ricker 1941; J. W. Cartwright, University of British Columbia, Vancouver, B.C., unpublished data; Thompson 1959). The extent of predation is not clear. For example, Ricker (1941) estimated consumption on the basis of quantities of food

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found in the stomachs of gillnet-caught fish, though it was not known how long the fish had been in the nets and how far digestion had proceeded. Thompson (1959) used gillnet samples to assess the role of squawfish as predators on salmon in the Lower Columbia River but, again, rates of digestion were not known. The studies reported in this paper were aimed at providing information on rates of digestion of squawfish of various sizes when fed on various quantities of fish, and held at various temperatures. Initially, experiments were conducted in field conditions at Griffen Lake, near Revelstoke, B.C. Subsequently, laboratory and field studies were done at Cultus Lake, near Chilliwack, B.C., with the assistance of the International Pacific Salmon Fisheries Commission. Additionally, observations were made at both lakes on the daily activity patterns of squawfish as indicated by net catches.

Feeding Activity

Griffen Lake — Squawfish were taken in a live trap set June 29, lifted September 4, 1968, and usually cleared of fish daily or more frequently. The net was set from shore with a lead of 40 m, and fished to a depth of 10 m. The total catch was approximately 5000 fish, from which a sample of 874 was examined for stomach contents. Food was found in the stomachs of 497 fish, and fish remains in 328. Those containing fish were above 12.5-cm fork length, and substantial quantities of fish were present only in squawfish over 17.5 cm. The proportion of squawfish containing fish in their stomachs (Y) was related to fork length (X) by the regression: $Y = 0.0211 + 0.0171X$. Insect remains predominated in squawfish under 17.5 cm.

The natural logarithm of average weight[1] of fish in the stomachs (Y) increased linearly with fork length (centimeters) of the squawfish (X) according to the relation $Y = -0.6940 + 0.0627X$. Additionally, a total of 85 spawning squawfish was examined and, almost without exception, their stomachs contained only trace amounts of food.

There was no significant difference in the average weight of fish in the stomach over the 24-h daily period, but the variability in weight was far greater at 2100 h

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[1] Measured volumetrically, specific gravity assumed to be 1.

TABLE 1. Results of analysis of stomach contents of northern squawfish (*Ptychocheilus oregonensis*) from Griffen Lake (July–September, 1967).

Food items	No. fish containing food items at different times					
	0900	1300	2100	0100	0500	Totals
Fish	269	34	4	4	17	328
Insect	39	11	0	2	28	80
Other	59	11	2	2	15	89
Empty	239	78	13	8	39	377
Total	606	134	19	16	99	874
Avg wt fish (*g*) and SD	3.47±.41	2.32±1.45	3.06±6.39	7.25±9.01	1.29±1.13	
% occurrence fish	44.39	25.37	21.05	25.00	17.17	
% occurrence insects	6.44	8.20	0.00	12.50	28.28	

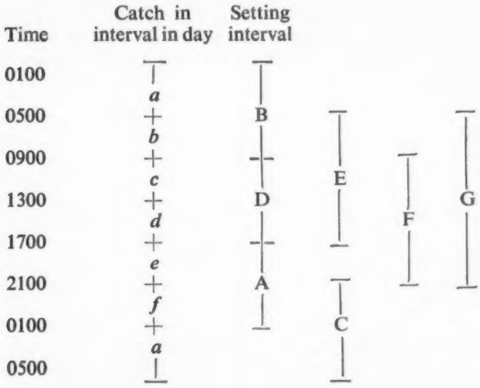
TABLE 2. Summary of the trap-catch data collected at Griffen Lake, August 20–25, 1967.

	Time														
	0100	*a*	0500	*b*	0900	*c*	1300	*d*	1700	*e*	2100	*f*	0100	*a*	0500
Aug. 20	:		$33(B_1)$		:		$3(D_1)$		:		$132(A_1)$		:		
Aug. 21	:		$84(B_2)$		:		$5(D_2)$		:		$74(A_2)$		:		
Aug. 22	:		$58(B_3)$		:			$26(F_1)$			:		$67(C_1)$		:
Aug. 23			:				$27(G_1)$				:		$18(C_2)$		:
Aug. 24			:			$21(E_1)$			:		$40(A_3)$		:		
Aug. 25	:		$37(B_4)$		:										

and 0100 h than at other times (Table 1), circumstantially suggesting that feeding occurs predominantly from dusk to dawn and the trap was catching fish during the feeding period.

If it can be assumed that greater activity was associated with feeding, and that trap catches reflected activity, it was evident from catches that feeding was occurring in the twilight and dark hours because overnight catches were almost invariably larger than those in daytime. To better delineate periods of activity, the trap was lifted at prescribed intervals on three occasions. July 26–28 the trap was lifted at 0500, 1300, and 2100 h; August 2–5 the lifts were at 0900, 1300, and 2100 h. In both cases the peak catch was at the morning lift.

Lifting of the trap at too frequent intervals appeared to disrupt the catching process, so to further pinpoint the hours of greatest catches a special pattern of setting was devised that enabled deduction of the average numbers of fish taken in different time periods. The intervals for recording the number in the trap, designated as capital letters, and 4-h time intervals in the day (small letters), may be represented as follows:



Replicates of setting intervals are denoted by subscripts (e.g. A_1, A_2, A_3). The sequence of the setting intervals over a 6-day period was:

Aug. 20————0100— B_1 —0900— D_1 —1700— A_1
Aug. 21— A_1 —0100— B_2 —0900— D_2 —1700— A_2
Aug. 22— A_2 —0100— B_3 —0900— F_1 —2100— C_1
Aug. 23— C_1 —0500— G_1 —2100— C_2
Aug. 24— C_2 —0500— E_1 —1700— A_3
Aug. 25— A_3 —0100— B_4 —0900

The catches in the various intervals are given in Table 2. This arrangement partitions the darker hours of the day into overlapping 4-h time periods. The solution for the determination of average catches in each period proceeds on the premise that all first deductions are used to provide average values before proceeding to second and further deductions; i.e. from Table 2 three estimates of catch in the interval *e* are available: F_1–D_1, F_1–D_2, G_1–E_1; and these are, respectively, 23, 21, and 6, with a mean $\bar{e} = 17$. Similarly, three estimates of *b* are available: E_1–D_1, E_1–D_2, G_1–F_1; and these are, respectively, 18, 16, and 1, with a mean $\bar{b} = 12$. From $\bar{b} = 12$ there are four estimates of catch in interval *a* available from subtracting $\bar{b}$ from each of the four B values, and these are, respectively, 23, 74, 48, and 27, for a mean $\bar{a}$ of 43. From ¯ three estimates of *f* are available from subtracting *e* from each of the A values, and the estimates of *f* are, respectively, 115, 57, and 23, for a mean $\bar{f}$ of 65. Finally, from E–$\bar{b}$ and F–$\bar{e}$ we can deduce $(c+d)$ as 9 and 9, and these values averaged with D_1 and D_2 (3 and 5) yield $(\bar{c} + \bar{d}) = 7$.

Thus, the average catches, and presumably the activity of fish at different times, can be summarized as in Table 3, with a peak at 2100–0100 h and most of the remainder of the catch at 0100–0500 h.

Cultus Lake — A trapnet was set throughout the period April 20–June 3, 1969, in Cultus Lake near the

TABLE 3. Deduced number of squawfish taken at various time periods at Griffen Lake.

Time period	Activity index
$\bar{a}$ = 0100–0500	43
$\bar{b}$ = 0500–0900	12
$\bar{c}+\bar{d}$ = 0900–1700	7
$\bar{e}$ = 1700–2100	17
$\bar{f}$ = 2100–0100	65

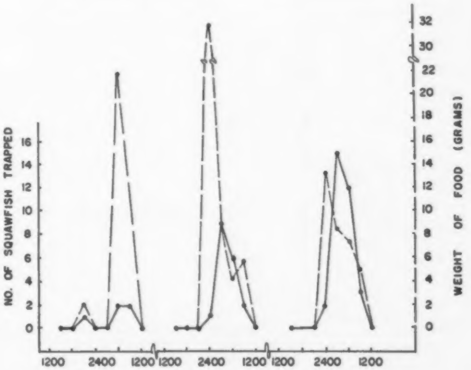


FIG. 1. Catch (dashed line) and weight of food in stomachs (solid line) of northern squawfish (*Ptychocheilus oregonensis*) from a trap net lifted at 3-h intervals from 1500 h onwards on April 25–26, May 1–2, and May 9–10, 1969, Cultus Lake.

outlet stream. On three occasions, April 25–26, May 1–2, and May 9–10, during the peak period of smolt migration, the trapnet was lifted at 3-h intervals. The greatest numbers of squawfish were caught between 2400 and 0600 h, and the mean volume of food in the stomachs was also highest at these times (Fig. 1). The squawfish were consuming sockeye salmon smolts almost exclusively, only 15% had empty stomachs, and the average number of smolts consumed was between two and three on all three occasions (mean number 2.50, mean weight 10.51 g, sample size 55). As in the case of the Griffen Lake experiments, peak feeding activity seemed to occur during the hours of darkness, in Cultus Lake the time of feeding no doubt reflecting in some measure the daily pattern of smolt migration.

Rate of Digestion

Griffen Lake — As a first digestion experiment, 164 squawfish ranging in size from 19.4- to 38.3-cm fork length (mean 24 cm) were force-fed 2.0 ± 0.1 g of redside shiner (*Richardsonius balteatus*). This quantity of food was somewhat below the average found in stomachs of fish from the trap, but was a convenient amount because of the size of the shiners and the rigors of force-feeding. The squawfish were held in herring mesh enclosures 2 × 2 × 3 m deep and starved for 72 h before feeding. Immediately prior to feeding, the fish were anesthetized with tricane methanesulfonate (Sandoz M.S. 222) at a concentration of 1–15,000. The residual stomach contents were pumped out at 6, 12, 18, or 24 h after feeding, the fish being anesthetized again for this operation. The stomach pump was modelled after that of Seaburg (1957), and the pumped residues were filtered through screen of 1-mm mesh before weighing to 0.1-g accuracy. During the course of the experiments the ambient water temperature varied between 17 and 20 C.

The rate of digestion was best described by a regression of log weight of food remaining[2] on time in

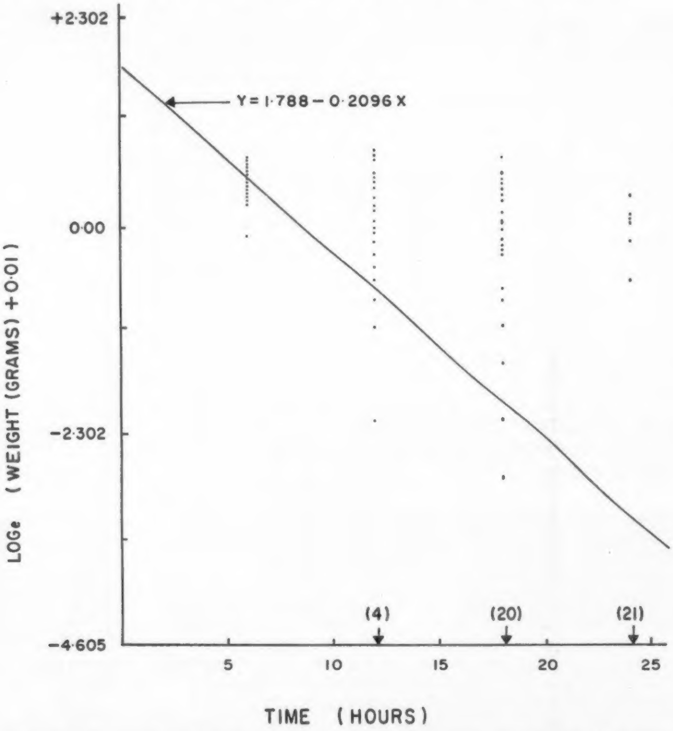


FIG. 2. Regression of natural logarithm of weight of food in stomach against time, for 164 squawfish fed 2.0 g of redside shiner (*Richardsonius balteatus*). Numbers of fish with empty stomachs given in brackets. Griffen Lake, June 1968.

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[2] In calculating this regression, 0.01 was added to each weight of food remaining, to avoid the generation of negative infinite log values.

hours (Fig. 2), but the wide scatter of the data suggested that the circumstances of the experiment were not adequately controlled. Among the contributing factors to the scatter were the variable length of the fish, varying temperature, the effects of the anesthetic, force-feeding and handling, and the use of wet weights. For simple regression, the instantaneous rate of digestion was 0.2096, or roughly 20%/h, but the intercept of the regression (1.788) was a poor estimate of the log initial weight fed (0.698). When the regression was constrained to go through the known log weight of food that was initially fed (0.698), the estimate of the rate of digestion was 0.148, corresponding to roughly 14%/h.

Cultus Lake — (*Field experiments*) — Approximately 650 squawfish were removed from the Cultus Lake live trap between 1200 May 1 and 1000 May 2. The fish were divided into five groups. One of these groups was killed and preserved immediately, and the other groups were sacrificed after 6, 12, 18, and 24 h, respectively. The temperature at the capture site and in the holding pens ranged from 10 to 12 C.

The stomach contents were almost exclusively sockeye salmon smolts. The natural logarithm of the average volume of stomach contents (Y) at the time of capture was significantly linearly related to the fork length of the fish (X) in a sample of 100 by the relation: $Y = -9.4130 + 0.4350X$. The regression of log volume of food in the stomach against time since capture was undertaken separately for each of 10 size-groups at 1-cm intervals from 24.5 to 34.5 cm. The slopes of the regressions were not significantly different,

TABLE 4. Regression statistics for the relation between $\log_e$ weight of food in the stomach (Y) and time in hours since capture (X) of squawfish of 10 ranges of fork length, Cultus Lake, May 1–2, 1969.

Fork length (*cm*)	Intercept	Slope	Sample size
24.6–25.5	0.9519	–0.1973	12
25.6–26.5	0.7191	–0.1384	28
26.6–27.5	–0.0762	–0.1504	42
27.6–28.5	–0.5731	–0.1245	82
28.6–29.5	–0.5381	–0.0847	104
29.6–30.5	0.6440	–0.1467	117
30.6–31.5	0.8899	–0.1439	97
31.6–32.5	1.6643	–0.1345	81
32.6–33.5	1.5736	–0.1464	50
33.6–34.5	1.9954	–0.1575	45

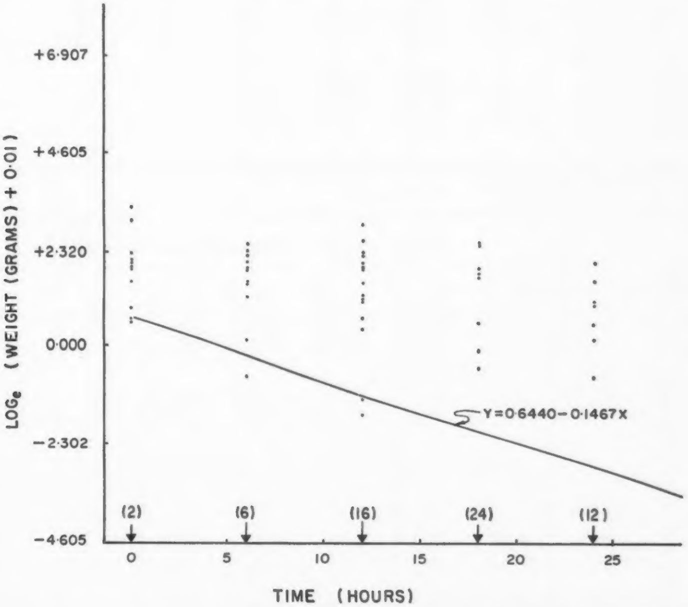

FIG. 3. Regression against time of the natural logarithm of weight of food in stomachs of 117 squawfish (29.6–30.5 cm) sacrificed at capture and at various times after capture. Numbers of fish with empty stomachs given in brackets. Cultus Lake, May 1–2, 1969.

and almost uniformly reflected a rate of digestion of about 14%/h for all size-groups. The intercepts were significantly different, reflecting the larger volumes of food in the stomachs of the larger fish (Table 4).

Inspection of the data suggests that the slope of the regression of log weight of food in the stomach against time since capture may not be entirely adequate as a measure of rate of digestion for individual squawfish. For all sizes of fish it was notable that at all times relatively few fish contained small quantities of food in their stomachs. Figure 3 is typical, and illustrates that, at later times, the proportion of fish with empty stomachs increased from 16% at time of capture, to 63% after 24 h (Table 5). The simplest explanation for these results is that the food is retained virtually intact in the stomach during early stages of digestion and is then rapidly liquefied and passed to the intestine. Nevertheless, the majority of squawfish have cleared

TABLE 5. Frequency of occurrence at time of capture and after various lengths of time, of empty stomachs and stomachs containing food in squawfish of various fork lengths. Data collected at Cultus Lake, B.C., May 1–2, 1969.

	Time since capture									
	0 h		6 h		12 h		18 h		24 h	
Fork length (*cm*)	Empty	With food	Empty	With food	Empty	With food	Empty	With food	Empty	With food
24.6–25.5	0	1	0	2	1	1	2	1	3	1
25.6–26.5	0	1	0	3	3	4	2	2	8	5
26.6–27.5	0	6	4	2	7	7	6	2	6	2
27.6–28.5	7	9	2	5	11	11	10	6	18	3
28.6–29.5	3	6	7	6	17	20	11	9	15	10
29.6–30.5	2	16	6	13	16	21	14	8	12	9
30.6–31.5	3	15	4	11	8	17	11	9	12	7
31.6–32.5	0	16	2	13	4	14	6	10	9	7
32.6–33.5	1	9	1	11	4	8	1	4	6	5
33.6–34.5	0	5	2	6	2	10	5	5	5	5
Totals	16	84	28	72	73	113	68	56	94	54

TABLE 6. Cultus Lake laboratory experiments on rate of digestion in squawfish. Each replicate included three fish, one at each of three volumes of food.

Temp range (*C*)	Size categories[a]	No. of replicates	Dissection time (hours)							
			4	6	8	10	12	14	18	24
4–6	1	2		x			x		x	x
	2	2		x			x		x	x
	3	2		x			x		x	x
	4	2		x			x		x	x
	5	2		x			x		x	x
	6	2		x			x		x	x
8–10	2	2		x			x		x	x
	3	4		x			x		x	x
	4	4		x			x		x	x
15–16	2	2		x			x		x	x
	3	4		x			x		x	x
	4	4		x			x		x	x
19–21	3	4	x	x	x	x	x	x		
24–25	3	4–5	x	x	x	x	x			

[a] Categories: 1 = Fork length (FL) 15.0–19.9 cm, 2 = (FL) 20.0–24.9, 3 = (FL) 25.0–29.9, 4 = (FL) 30.0–34.9, 5 = (FL) 35.0–39.9, 6 = (FL) >40.0.

TABLE 7. Regression statistics for relation between weight of food in the stomach and time since feeding for squawfish of various fork lengths held at various temperatures, Cultus Lake, June 1969. (Asterisk denotes significance at $\alpha = .05$.)

			Temp 6 C			Temp 10 C			Temp 15 C			Temp 20 C			Temp 24 C		
Fork length (*cm*)	Wt fed	Log (W +.01)	Sample size	Intercept	Slope	Sample size	Intercept	Slope	Sample size	Intercept	Slope	Sample size	Intercept	Slope	Sample size	Intercept	Slope
15.0–19.9																	
Pooled			21	0.7692	−0.03683												
Low	0.87	−0.128	8	0.5771	−0.02787*												
Medium	1.17	0.165	6	1.889	−0.1351												
High	3.46	1.244	7	1.271	−0.008517												
20.0–24.9																	
Pooled			21	1.060	−0.03728	22	1.369	−0.1236*	21	1.511	−0.02413*						
Low	1.22	0.207	8	0.2780	−0.03897	8	0.1971	−0.1212*	6	−1.162	−0.1501*						
Medium	2.39	0.875	8	0.7576	0.001719	8	1.874	−0.1511*	8	1.96	−0.2760*						
High	4.79	1.569	5	1.448	0.004693	6	2.077	−0.07777*	7	2.442	−0.2216*						
25.0–29.9																	
Pooled			23	1.330	−0.04367	45	1.052	−0.06710*	45	1.674	−0.2412*	68	1.993	−0.4143*	67	1.920	−0.4869*
Low	1.60	0.476	8	1.100	−0.08537*	16	0.08154	−0.08945*	16	−0.362	−0.1784*	24	0.2975	−0.3705*	22	0.4854	−0.4542*
Medium	3.19	1.163	8	1.180	−0.02745	15	1.726	−0.1103*	13	1.756	−0.2477*	24	2.574	−0.4504*	22	2.621	−0.5441*
High	6.39	1.860	7	1.913	−0.02492*	14	1.941	−0.03335*	16	3.647	−0.2994*	20	3.519	−0.4440*	23	3.025	−0.5116*
30.0–34.9																	
Pooled			23	1.551	−0.03576	45	1.246	−0.05744*	46	2.151	−0.02652*						
Low	2.00	0.698	8	0.8516	−0.04694	16	0.7458	−0.09576*	16	0.2923	−0.213*						
Medium	4.00	1.389	7	1.669	−0.04303	16	1.457	−0.05723*	15	2.586	−0.2775*						
High	8.00	2.081	8	2.169	−0.01917*	13	2.352	−0.06020*	15	3.539	−0.2990*						
35.0–39.9																	
Pooled			24	2.003	−0.06558*												
Low	2.42	0.888	8	1.801	−0.1098*												
Medium	4.84	1.579	8	1.715	−0.4174												
High	9.68	2.271	8	2.493	−0.04514												
>40.0																	
Pooled			20	3.906	−0.1403												
Low	2.86	1.054	7	1.815	−0.1201												
Medium	5.73	1.747	8	2.533	−0.09301*												
High	11.44	2.438	5	6.268	−0.08294												
Sizes pooled																	
Pooled				1.751	−0.05896*		1.169	−0.07247*		1.838	−0.2511*		1.993	−0.4143*		1.920	−0.4869*
Low				0.9285	−0.06574*		0.3704	−0.09834*		−0.1876	−0.1902*		0.2975	−0.3705*		0.4854	−0.4542*
Medium				1.554	−0.04937*		1.635	−0.09598*		2.119	−0.2653*		2.574	−0.4504*		2.621	−0.5441*
High				2.693	−0.04494		2.052	−0.04718*		3.385	−0.2859*		3.519	−0.4440*		3.025	−0.5116*

TABLE 8. Statistics for regression of weight of food in stomach and time after feeding when regression constrained through the known weight of food fed, Cultus Lake, June 1969. (Asterisk denotes significance at $\alpha = .05$.)

Fork length (*cm*)	Log (W_t+.01)	6 C		10 C		15 C		20 C		24 C	
		Sample size	Slope	Sample size	Slope	Sample size	Slope	Sample size	Slope	Sample size	Slope
15.0–19.9											
Pooled		21									
Low	–0.128	8	–0.01756*								
Medium	0.165	6	–0.05748								
High	1.244	7	–0.007057*								
20.0–24.9											
Pooled		21		22		21					
Low	0.207	8	–0.03502*	8	–0.1218*	6	–0.2298*				
Medium	0.875	8	–0.005057	8	–0.09591*	8	–0.2157*				
High	1.569	5	–0.004802	6	–0.04818*	7	–0.1687*				
25.0–29.9											
Pooled		23		45		45		68		67	
Low	0.476	8	–0.05067*	16	–0.1113*	16	–0.2249*	24	–0.3878*	22	–0.4532*
Medium	1.163	8	–0.02649*	15	–0.07778*	13	–0.2114*	24	–0.3133*	22	–0.3855*
High	1.860	7	–0.02185*	14	–0.02879*	16	–0.1999*	20	–0.2859*	23	–0.3838*
30.0–34.9											
Pooled		23		45		46					
Low	0.698	8	–0.03841*	16	–0.09311*	16	–0.2355*				
Medium	1.389	7	–0.02795*	16	–0.05343*	15	–0.2121*				
High	2.081	8	–0.01425*	13	–0.04588*	15	–0.2149*				
35.0–39.9											
Pooled		24									
Low	0.888	8	–0.05912*								
Medium	1.579	8	–0.03415*								
High	2.271	8	–0.03280*								
>40.0											
Pooled		20									
Low	1.054	7	–0.06125*								
Medium	1.747	8	–0.04936*								
High	2.438	5	+0.1435								

their stomach contents within 24 h, the median point lying between 12 and 18 h.

Laboratory experiments — To determine the effect of temperature, volume of food, and size of fish on the rate of digestion, squawfish of various sizes were force-fed known volumes of food and sacrificed after various intervals of time. It was not possible to do all combinations of treatments in a factorial design; Table 6 summarizes what was undertaken. Trapped squawfish were held in a large rearing channel (11–13 C) and, prior to the experimental period, were fed ad lib. on redside shiners (*R. balteatus*). The evening prior to a feeding experiment, squawfish were transferred to plywood controlled temperature tanks 0.6 × 2.6 × 0.4 m deep. After 12 h, they were force-fed one redside shiner, and they were then starved for 72 h, at which time they were force-fed a prescribed weight of juvenile (3–6 cm) hatchery rainbow trout (*Salmo gairdneri*). At a prescribed time after the feeding the squawfish were killed, the stomach contents removed, washed over screening of 1-mm mesh, damp dried, and quick frozen for subsequent weighing.

The weights of food fed were arbitrarily set at low (2 g) medium (4 g), and high (8 g) for fish of 32.5-cm fork length, a range that spanned that observed at Griffen Lake in the stomachs of trapped fish (for 32.5-cm fish the mean weight of stomach contents was 3.84 g). For fish of other sizes the weight of food was chosen to be proportional to the 0.44 power of weight of the fish. Although this rule is contrary to the generally accepted rule that ration is proportional to the two-thirds power of weight, it provided rations that approximated what were observed in the field at Griffen Lake (e.g. for a fish of 12.5-cm fork length, 1.06 g; 32.5-cm, 3.84 g; 40-cm, 6.14 g). The weights of food in the stomachs of trap-caught fish at Cultus Lake were also in the same range as those fed in the laboratory. For example, size 3 fish (25.0–29.9 cm) taken in the field contained an average stomach content of 3.76 g of food, slightly more than the medium ration in the laboratory experiment.

The results are summarized in Table 7, giving slopes and intercepts for standard regression, and Table 8, slopes for regressions constrained through the known weight of food fed. Contrasting the results in the two tables, standard regression techniques usually overestimated the amount of food fed (intercept greater than known weight fed) and a higher estimate of the rate of digestion than constrained regression. Apparently, this results from an initial period of hydrolysis of the food, which increases its weight, followed by a period in which the food is retained in the stomach during early stages of digestion, and is then followed by a short period of relatively fast evacuation of most of the stomach contents. Because the period of observation ended shortly after small residues remained, the initially quantity of food fed was overestimated. To a lesser degree the average rate of digestion was also overestimated.

Despite the differences in the two analyses, they both show three major effects: (1) the rate of digestion substantially increases with temperature, from about 5%/h at 4–6 C, to 40–50%/h at 24 C; (2) larger weights of food are digested at slower rates; and (3) there are no consistent differences in the rate of digestion by different sizes of fish. Figure 4 illustrates the substantial increase in rate of digestion with increased temperature for size 3 fish, and the consistently higher rates of digestion of smaller quantities of food.

Discussion

The foregoing results clearly indicate that squawfish feed most intensively in the hours of dusk and darkness, and that an average meal is usually evacuated from the stomach within 24 h. The ambient temperature where squawfish are most commonly taken in nets approximates 10–15 C at which digestion rates for various volumes of food

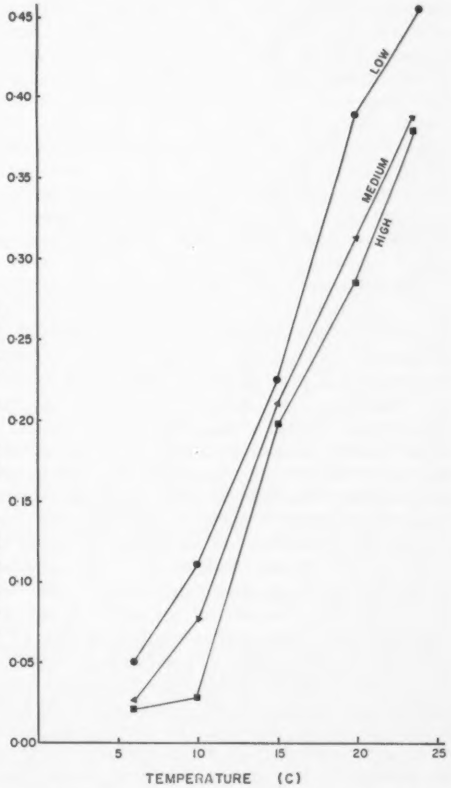

FIG. 4. Instantaneous hourly rate of digestion in stomachs of squawfish of fork length 25.0–29.9 cm at various temperatures for low (1.60 g), medium (3.19 g), and high (6.39 g) levels of food.

are in the range of 5–20%/h (Table 8). This rate is not dissimilar to that for bluegill sunfish (*Lepomis macrochirus*) at 18–21 C, as calculated from the results of Windell (1967, figure 1), for which several kinds of foods were digested at approximately 13 %/h. The stomachs of bluegill sunfish were usually empty from 17–19 h after a natural feeding.

All the rates of digestion here reported for squawfish are much higher than those given by Molnar et al. (1967) for various species of predatory fishes of comparable size and at comparable temperature, although in Molnár's work the size of fish used as food was larger — sufficient to fill the stomach. For example, at 10 C, gastric digestion in largemouth bass (*Micropterus salmoides*) was not completed for 50 h. The digestion rates in excess of 40%/h for squawfish held at 24 C in our laboratory experiments are extraordinary, but were observed consistently for various weights of food and were part of a trend to higher rates at higher temperatures. They are far in excess of rates reported by Molnár et al. (1967); for example, at 25 C pikeperch (*Lucioperca lucioperca*), Eurasian perch (*Perca fluviatilis*), and largemouth bass (*M. salmoides*) did not achieve 100% gastric digestion until 28, 21, and 19 h, respectively. The change in rate of digestion with temperature is comparable to that reported by Molnár et al. for pike, perch, and the European catfish (*Silurus glanis*).

The findings of this study have implications for field assessment of total consumption of fish by squawfish. For example, if squawfish are left in gillnets for extended periods after they are captured, the quantity of food in the stomach when the nets are lifted may be a poor indicator of what they take as an average meal. Without knowledge of when the fish were caught, and the length of time that squawfish remain alive (and digesting) in nets after capture, it is difficult to be precise about what "correction factor" might be applied, but it is obvious that estimates of consumption based on gillnet-caught fish are minimal. Thus, the estimates of Ricker (1941), in some years based on fish that had been taken in nets set for 48–72 h, are undoubtedly low. Even when nets were set for 24 h, it would be anticipated that substantial digestion would have occurred, and though Ricker was aware of this source of error, it would appear to have been greater than he appreciated. The lower temperatures of the early spring period when squawfish predation on sockeye fry and smolts is intense, are in some measure associated with slower digestion rates, but even at 4–6 C, the rate of digestion approximates 3%/h and enables two-thirds evacuation of the stomach within 36 h.

Average rates of digestion may be misleading. In laboratory studies, standard regression of log weight of food fed against time overestimated the amount of food fed, reflecting an initial period of hydrolysis followed by a fast rate of evacuation of most of the stomach contents. The same phenomenon would account for the field observations of digestion rate, for which the average time to evacuation of the stomach was more meaningful than the average digestion rate. Under field conditions, with ambient temperatures of 10–12 C, and with feeding natural, two-thirds of the squawfish had empty stomachs after 24 h.

The merits of control of predators such as squawfish, for increasing production of sockeye salmon, have been a matter of anecdotal remark for many years. The estimates of Ricker (1941) and Foerster and Ricker (1941) indicated that, because of the quantity of prey taken, squawfish removal programs could be profitable. This study suggests that the total consumption of sockeye by squawfish could be even larger than these authors estimated, and the benefits of control programs might thus be accordingly greater.

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Life Cycles of Three Limnetic Copepods in a Beaver Pond

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CARTER, J. C. H. 1974. Life cycles of three limnetic copepods in a beaver pond. J. Fish. Res. Board Can. 31: 421–434.

In a small, shallow beaver pond near Georgian Bay, Ontario, life histories of each of the three limnetic copepods found were basically similar in the 3 yr 1970–72 although the patterns were occasionally obscured. *Cyclops bicuspidatus thomasi* presumably emerged from diapause at stage C 4 when the ice broke and went through one complete cycle to C 4 in 3–4 wk, reentering diapause in late spring. *Mesocyclops edax* emerged from diapause at C 4 slightly later in spring and underwent four complete cycles, reentering diapause in midautumn; normal time to complete a generation was about 1 mo. *Diaptomus reighardi* hatched from resting eggs in early May and produced four complete generations, the last maturing in early autumn; development of spring and summer generations required 1 mo and that of autumn generations about 1½ mo. During 1972 lowered water levels resulted in increased heat content of the pond per unit volume and this was reflected in some acceleration of development rates of *M. edax* and *D. reighardi*. At most sampling times *M. edax* had about double the fecundity of *D. reighardi* indicating a correspondingly high mortality rate that might be the result of lower egg viability, cannibalism, or competition between nauplii of the two species.

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Le cycle vital de chacun des trois copépodes limnétiques trouvés dans un petit étang de castors, près de la baie Georgienne, Ontario, a été fondamentalement le même durant les 3 années 1970–72, bien que les modalités en aient été parfois obscurcies. *Cyclops bicuspidatus thomasi* émerge vraisemblablement de la diapause au stade C 4, au moment de la rupture des glaces, et passe par un cycle complet jusqu'au stade C 4 de la seconde génération en l'espace de 3–4 semaines, rentrant dans la diapause tard au printemps. *Mesocyclops edax* émerge de la diapause à C 4 un peu plus tard au printemps et passe par quatre cycles complets, rentrant dans la diapause à la mi-automne; la durée normale du développement d'une génération est d'environ 1 mois. *Diaptomus reighardi* émerge d'oeufs dormants au début de mai et produit quatre générations complètes, la dernière atteignant la maturité au début de l'automne; le développement des générations de printemps et d'été requiert 1 mois, et celui des générations d'automne, environ 1½ mois. En 1972, une baisse du niveau de l'eau a causé une augmentation du contenu calorifique de l'étang par unité de volume, qui, à son tour, a entraîné une accélération des taux de développement de *M. edax* et *D. reighardi*. La plupart du temps, la fécondité de *M. edax* est à peu près le double de celle de *D. reighardi*, indiquant un taux de mortalité également élevé, résultat possible d'une viabilité plus faible des oeufs, cannibalisme ou concurrence entre les deux espèces au stade nauplius.

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LIFE histories of limnetic copepods are still inadequately known. This is due partly to the necessity of collecting samples at relatively short intervals over at least one season and partly to the extreme difficulty of identifying younger instars when more than one cyclopoid copepod or one calanoid copepod coexist. A further problem involves accurate interpretation of the data to determine whether successions of instar pulses through time represent successive generations, i.e. that each is on the one hand the progeny of the immediately prior generation and on the other the parents of that immediately following; however, this problem is greatly reduced if a life history is followed over a number of years and the same generations are found to occur at similar times.

This report elucidates the life histories and certain interspecific relationships during 3 yr 1970–72 of *Cyclops bicuspidatus thomasi* S. A. Forbes, *Mesocyclops edax* (S. A. Forbes), and *Diaptomus reig-*

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hardi Marsh, the three limnetic copepods of Black Pond, one of many small, shallow, fishless beaver ponds of the Georgian Bay coast (Carter 1971). The waters of these ponds are usually highly acid and exclude most native limnetic crustaceans whereas those species which can tolerate the extreme conditions usually build up large seasonal populations.

Black Pond (45°32′N, 80°27′W) is situated on a large island near the western shore of Georgian Bay, Ont. and was impounded from the Bay by a beaver dam of uncertain age. It is usually ice-free from late April or early May until late November. Prior to September, 1972 it had a pH of about 5.0, a specific conductance of about 28 μmho/cm, and a color of about 200 Pt units. Further details regarding water chemistry and other zooplankton are found in Carter (1971). The pond has an area of 1.13 hectares and consists of two narrowly connected basins (Fig. 1). Normal maximum depth following spring breakup was 1.9 m, at a point close by the dam in the outer basin. In both 1970 and 1971 the water level was maximal in early May and then dropped gradually until late autumn,

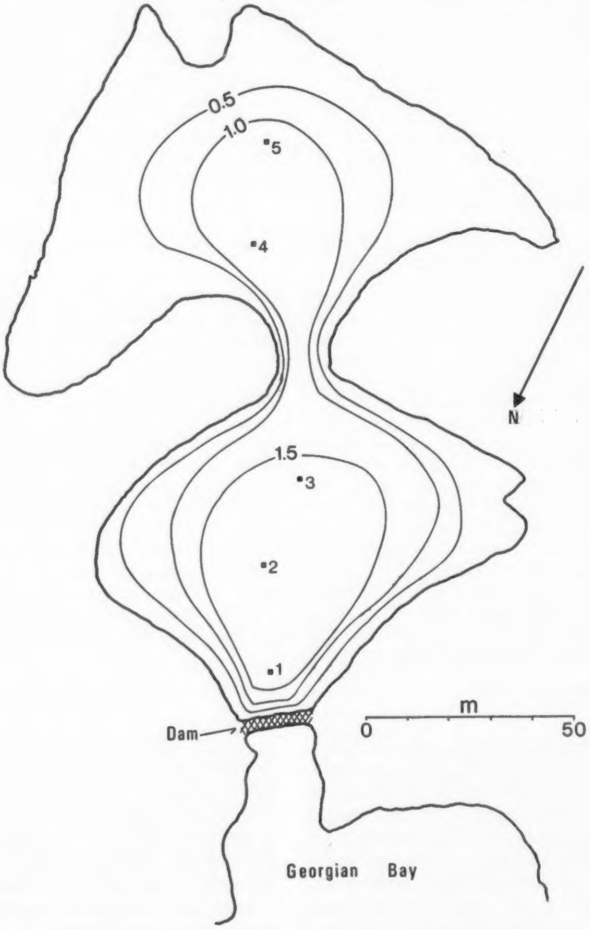

FIG. 1. Bathymetry and sampling stations of Black Pond. Depth contours in meters.

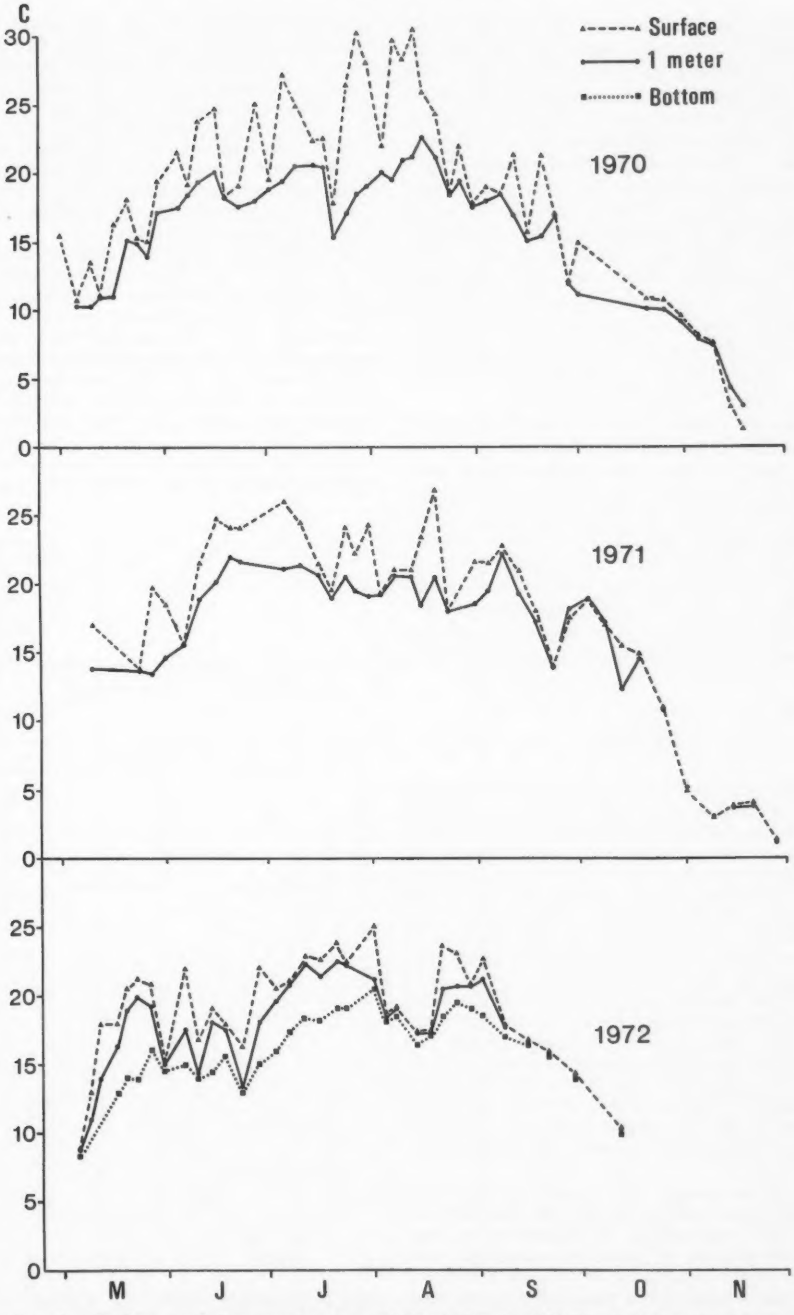

FIG. 2. Surface and 1-m temperatures of Black Pond, 1970–72, and bottom (about 1.6 m) temperatures, 1972, of Black Pond.

when it was about 25 cm lower than in spring. At breakup in 1972 the dam sprang a leak and the water level was about 50 cm below the early spring normal. The leak was soon repaired by hand but the water level dropped another 10 cm during the next 6 wk due to persistent dry weather. Until mid-September the level rose slightly as a result of successive torrential rains; by then it was the same height as in the previous spring but was still about 25 cm below the seasonal normal. Meanwhile the waters of Georgian Bay, which had been rising about 20 cm annually since 1965, had crept near to the top of the dam. At some point between September 15 and 21 the dam was breached from outside, the pond became flooded with hard Georgian Bay waters, and by mid-October the indigenous crustacean plankton were almost extinct.

Because of its shallow depth Black Pond tended to heat rapidly in spring and cool rapidly in autumn (Fig. 2). Also, the pond was protected from most winds by its small area, the granite banks on most sides, and the relatively thick vegetation on the surrounding land. Consequently each summer consisted of calm intervals with rapid heating at the surface alternating with periods of wind-induced vertical mixing. Bottom temperatures were recorded in 1972 only, at about 1.6 m, and these were almost always lower than those at 1 m except during autumn.

Methods

Five permanent stations were established along the long axis of the pond, three in the deeper outer basin and two in the shallower inner basin (Fig. 1). Samples were collected with a Schindler-Patalas plankton trap of 23 liters capacity equipped with a net and collecting bucket of #25 mesh (apertures 0.064 mm) commencing each year soon after breakup in early May and terminating with freeze-up in November in 1970–71 and with flooding in October 1972. Between May and early autumn, samples were taken usually every 3–5 days; during autumn, usually every 5–8 days. A single winter visit was made in February, 1972 when the pond was covered with 30 cm of ice. Two samples were taken on each date at each station to represent the water column, the first with the top of the trap 15 cm below the surface and the second with the bottom about 20 cm above the sediment. The 10 samples were then combined into a single pond sample and preserved in 10% formalin.

In the laboratory each pond sample was diluted to a suitable volume, usually between 500 and 2000 ml, and after thorough mixing, 25-ml subsamples were extracted for analysis. At least four subsamples were analyzed from each sample and at least 700 individuals and 500 eggs of each species were counted from each sample when populations were sufficiently large. In samples where instars or eggs of a species were scarce, successive subsamples were analyzed until the numbers counted appeared to indicate the population structure. Cephalothorax lengths of at least 50 adult female *D. reighardi* from each sample were measured with an occular micrometer. For nauplii of *C. b. thomasi* and *M. edax*, instars were not determined since equivalent stages of the two species are indistinguishable in rapid field counts, and because N 4 and N 5 of both species are separable only by mounting and analysis under a compound microscope.

To determine comparative egg development rates of *D. reighardi* and *M. edax* live plankton samples were taken to the laboratory during July and August, 1970. Adult females without eggs and males were anesthetized with saturated chloretone solution and transferred to 25-ml vials, about 20 females and a few males to each vial. The vials were then maintained in incubators at various temperatures between 5 and 31 C. Periodic observations were made and when egg sacs appeared these were removed to 10-ml vials and maintained at the same temperatures. Egg sacs were inspected regularly and the hatching times of the eggs recorded.

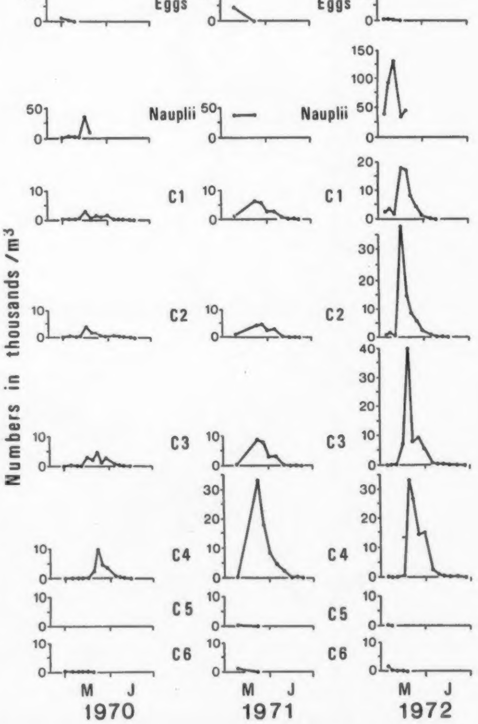

FIG. 3. Seasonal cycles of *Cyclops bicuspidatus thomasi* in Black Pond 1970–72.

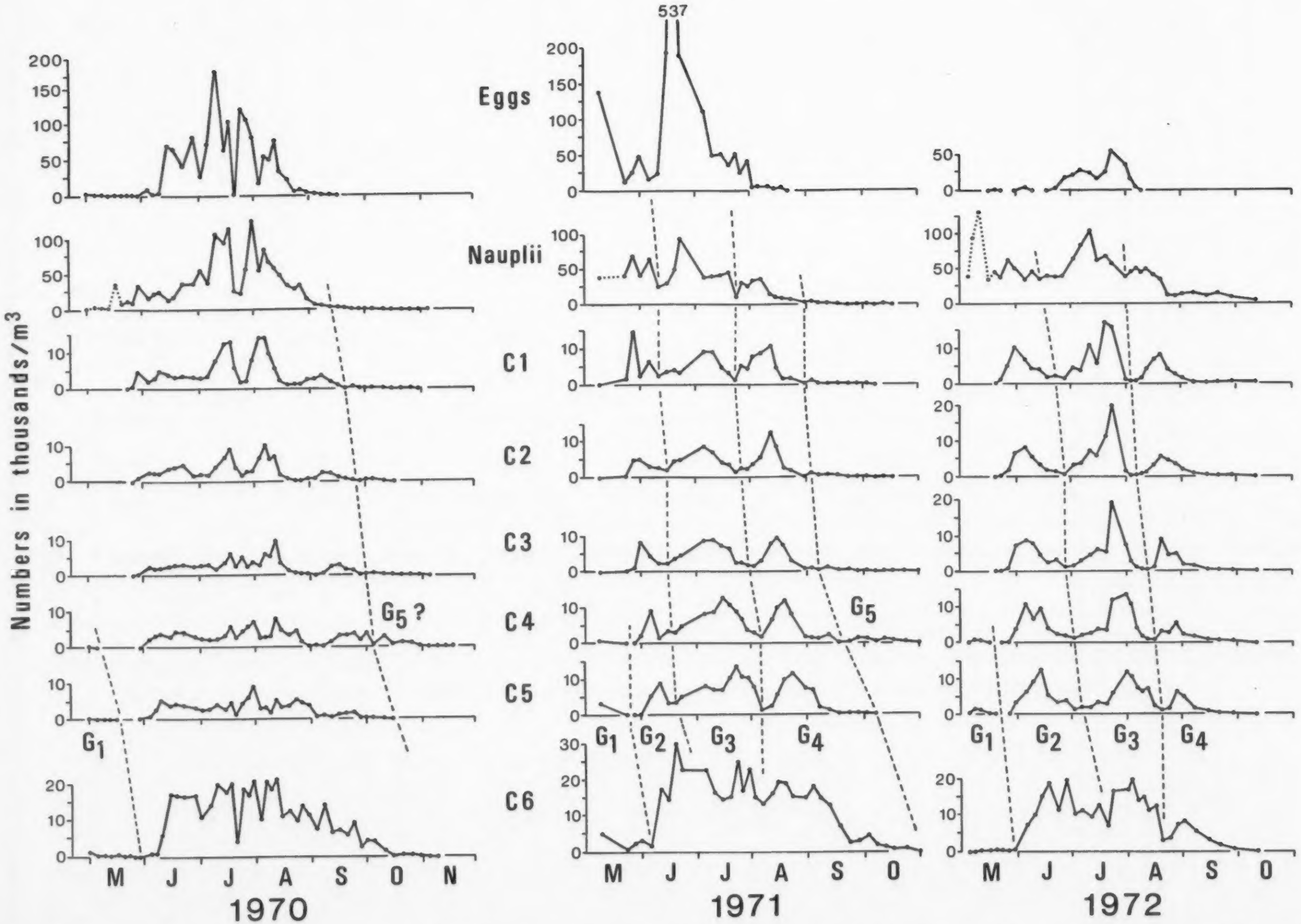

FIG. 4. Seasonal cycles of *Mesocyclops edax* in Black Pond, 1970–72. Broken lines represent estimated generation limits. Dotted lines indicate nauplii probably belonging to *C. b. thomasi.*

TABLE 1. Copepodite instar medians and intervals (days) between successive medians for *M. edax* in Black Pond, 1971–72.

	1971						1972						
	G_2		G_3		G_4		G_2		G_3		G_4		
Instar	Median	Duration	Median	Duration	Median	Duration	Median	Duration	Median	Duration	Median	Duration	Mean duration
C 1	May 29		July 4		Aug. 8		June 4		July 20		Aug. 20		
		3.3		1.4		3.7		2.6		2.2		3.8	2.8
C 2	June 2		July 5		Aug. 12		June 6		July 22		Aug. 24		
		1.8		2.6		3.8		1.9		2.7		1.0	2.3
C 3	June 3		July 8		Aug. 15		June 8		July 24		Aug. 25		
		3.6		6.1		4.1		2.6		3.7		4.2	4.1
C 4	June 7		July 14		Aug. 19		June 11		July 28		Aug. 30		
		3.8		4.6		4.6		2.7		4.2		2.5	3.7
C 5	June 11		July 19		Aug. 24		June 13		Aug. 1		Sept. 1		
C 1–C 5		12.5		14.7		16.2		9.8		12.8		11.5	12.9

Results

Cyclops bicuspidatus thomasi

Cyclops bicuspidatus thomasi was the first copepod present in large numbers in the pond each spring and presumably emerged from diapause immediately after the ice departed and the waters became oxygenated. When samples were first taken each year, egg-bearing females of the first (overwintered) generation were present and some of the second generation had already reached the younger copepodite stages (Fig. 3). The latter peaked at C 4 about May 20 after which the population rapidly declined. By early June most of the copepodites had disappeared and by mid-June only a few scattered individuals remained. Although the spring emergence was not observed it is assumed that C 4 is the only diapause stage as the second generation was not found to advance beyond this instar in the year of hatching; also, since there was never any

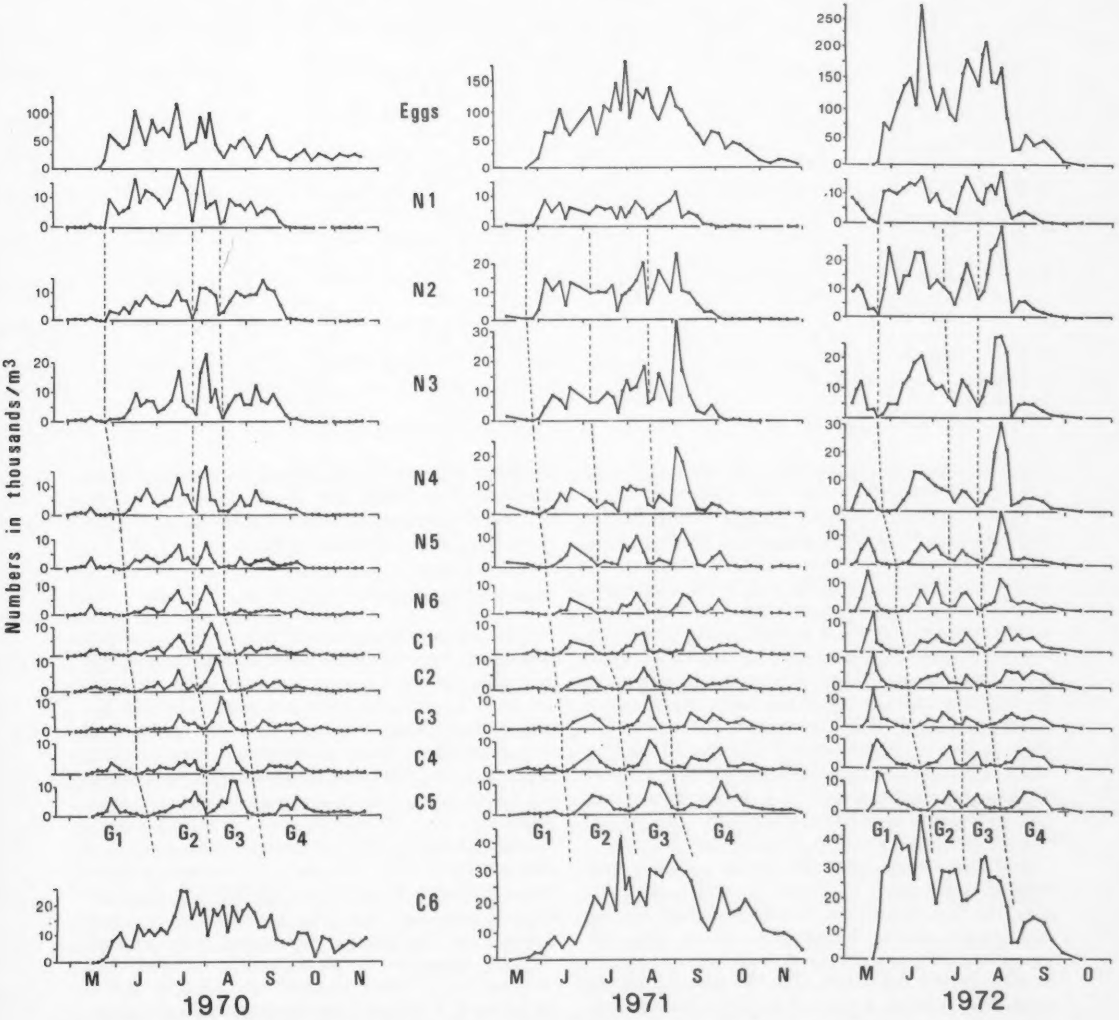

FIG. 5. Seasonal cycles of *Diaptomus reighardi* in Black Pond, 1970–72. Broken lines represent estimated generation limits.

TABLE 2. Instar medians and intervals (days) between

	1970						1971			
	G_2		G_3		G_4		G_2		G_3	
Instar	Median	Duration	Median	Duration	Median	Duration	Median	Duration	Median	Duration
N 1	June 29		Aug. 1		Sept. 2		June 16		July 26	
		0.7		3.5		5.2		2.0		3.8
N 2	June 29		Aug. 4		Sept. 7		June 18		July 30	
		4.3		–0.5		0.4		4.8		1.7
N 3	July 4		Aug. 4		Sept. 8		June 22		July 31	
		2.5		–0.4		2.7		3.1		1.8
N 4	July 6		Aug. 3		Sept. 10		June 26		Aug. 2	
		2.6		0.6		2.5		0.2		1.7
N 5	July 9		Aug. 4		Sept. 13		June 26		Aug. 4	
		3.4		0.3		1.8		1.2		1.5
N 6	July 12		Aug. 4		Sept. 15		June 27		Aug. 6	
		–0.5		2.9		2.2		0.8		2.0
C 1	July 12		Aug. 7		Sept. 17		June 28		Aug. 8	
		0.3		3.2		3.5		4.7		2.2
C 2	July 12		Aug. 10		Sept. 20		July 2		Aug. 11	
		3.1		3.7		5.3		1.8		3.0
C 3	July 15		Aug. 14		Sept. 26		July 4		Aug. 14	
		1.8		3.5		3.4		1.2		2.1
C 4	July 16		Aug. 17		Sept. 29		July 5		Aug. 16	
		4.8		3.2		8.1		4.1		3.0
C 5	July 21		Aug. 21		Oct. 7		July 9		Aug. 19	
C 1–C 5		10.0		13.6		20.3		11.8		10.3
N 1–C 5		23.0		20.0		35.1		23.9		22.8

apparent reduction in numbers between C 3 and C 4, it is improbable that C 3 was a diapause stage.

In 1972 the ice left Black Pond probably in late April and by May 27 the large C 4 mode of May 20 had declined to less than half; the situation was apparently similar in 1970 and 1971. Therefore, *C. b. thomasi* must have undergone each year one complete cycle, from C 4 to C 4, in approximately 3–4 wk. Ewers (1930) estimated an interval of about 4 wk between egg and adult at room temperature. In Leavenworth County State Lake, Kansas, Armitage and Tash (1967) suggested a complete life cycle in spring required about 35 days. In Marion Lake, B.C., McQueen (1969) estimated a time of 45 days between egg and C 4 during late spring and early summer, when temperatures were somewhat higher than those in Black Pond.

Although water temperatures in autumn were roughly equivalent to those in spring and *M. edax*, the obvious cyclopoid competitor, had become increasingly scarce during this period (Fig. 4), an autumn bloom of *C. b. thomasi* never occurred. In a pond near Kingston, Ont. Watson and Smallman (1971) found a second cycle in autumn and determined experimentally that daylength and temperature were the two critical factors controlling seasonal occurrence. In Black Pond, however, prevailing conditions were apparently not appropriate for an autumn cycle.

Mesocyclops edax

The seasonal cycle of *M. edax* shows certain differences between years but the basic pattern was probably the same during the three years (Fig. 4). In 1970 generations were not separable and the picture was mainly unclear. In 1971, however, it was possible to trace development through four distinct cycles. The first (overwintered) generation (G_1) had emerged from diapause by the first sampling date, May 10, and was present mainly as egg-bearing adults. The second generation (G_2) matured between early June and mid-July, producing enormous numbers of eggs (max 537,000/m^3), the third (G_3) in late July, and the fourth (G_4) in early September. Although a subsequent pulse of eggs did not appear in the plankton it is assumed that the fifth (overwintering) generation (G_5) developed as far as C 4, where it was represented by shallow pulses in late October and November. C 4 probably successive medians for *D. reighardi* in Black Pond, 1970–72.

		1972								
G_4		G_1		G_2		G_3		G_4		Mean duration
Median	Duration	Median	Duration	Median	Duration	Median	Duration	Median	Duration	
Sept. 2		May 8 (estimate)		June 15		July 23		Aug. 16		
	0.8		2.0		2.0		–1.1		0.3	1.9
Sept. 2		May 10		June 16		July 22		Aug. 16		
	1.8		1.8		5.1		0		1.3	2.1
Sept. 4		May 12		June 22		July 22		Aug. 17		
	2.1		2.1		3.2		0.1		1.2	1.8
Sept. 6		May 14		June 25		July 22		Aug. 18		
	3.1		2.4		2.3		0.3		0.6	1.6
Sept. 9		May 16		June 27		July 22		Aug. 19		
	3.4		0.9		3.0		0.3		3.5	1.9
Sept. 13		May 17		June 30		July 23		Aug. 22		
	5.9		2.7		1.6		1.2		7.6	2.6
Sept. 19		May 20		July 2		July 24		Aug. 30		
	6.0		0.9		1.1		0.8		5.8	2.9
Sept. 25		May 21		July 3		July 25		Sept. 5		
	3.9		1.7		3.4		1.7		–2.2	2.5
Sept. 29		May 22		July 6		July 26		Sept. 3		
	2.0		3.7		3.4		4.6		2.0	2.8
Oct. 1		May 26		July 9		July 31		Sept. 4		
	5.5		1.9		1.4		1.2		2.9	3.6
Oct. 6		May 28		July 11		Aug. 1		Sept. 7		
	17.4		8.2		9.3		8.3		8.5	11.8
	34.5		20.1		26.5		9.1		23.0	23.7

was the only diapause instar since C 5 was absent from all late autumn samples and C 1–C 3 were absent from all the earliest spring samples. During the early part of 1972 the pattern was similar to that in 1971. However, the third and fourth generations were both delayed somewhat, G_3 maturing only in late July and G_4 in late August. A shallow pulse of nauplii in September suggested that a fifth generation commenced but whether any animals reached C 4 and entered diapause is questionable since the pond began flooding later that month.

Computing the medians of instar pulses from the estimated generation boundaries (Fig. 4) suggested in most cases the intervals in time from the point when half of the animals in a generation had passed through a particular preadult copepodite to the point when half had passed through the next (Table 1).

No analysis was made of either the 1970 data or G_1 of 1971 and 1972. Mean C 1–C 5 duration of the remaining generations was 12.9 days. If 5 days are allowed for the interval C 5–C 6 and 2–3 days for egg development (Table 3) and if, as seems probable, total naupliar development rates were somewhat faster than those for preadult copepodites, then *M. edax* had a life cycle of approximately 1 mo.

In 1972 C 1–C 5 development time of each of G_2, was considerably shorter than for the same generations in 1971. This might be expected, following McLaren's (1963) hypothesis that adult size and generation length are negative functions of temperature, since the lowered water levels of 1972 brought the warmer surface waters closer to the bottom and increased the pond's mean heat content per unit volume. Despite these shorter copepodite development times, which probably indicate shorter generation development times, both G_3 and G_4 of 1972 occurred later in the summer than their counterparts of 1971 (Fig. 4). The apparent cause was the long delay in the appearance of G_3 in 1972 when a naupliar pulse did not form until mid-July, about 3 wk later than in 1971. Consequently by the time G_4 began in 1972 it was already about 2 wk behind G_4 of 1971.

The life history of *M. edax*, in Black Pond, involving four complete cycles from C 4 of the first (overwintered) generation to C 4 of the fifth (overwintering) generation, is apparently not general. In Severson Lake, Minn., Comita (1972) was able to trace only three distinct generations, the first emerging from diapause at C 4 in spring followed by two complete generations in summer.

Diaptomus reighardi

Though adult, egg, and early-nauplius pulses were generally not distinct, *Diaptomus reighardi* produced four generations in each of the 3 yr studied (Fig. 5). That the general indistinctness of generation boundaries in the adults was associated with overlapping of the generations is supported by the mean cephalothorax lengths. Instead of being marked by successive periods of constant values, the lengths generally decreased from May until midsummer, after which they showed little change (Fig. 6).

The first generation appeared in early May, presumably from resting eggs that overwintered on the bottom; on the one occasion when winter samples were collected through the ice no free-swimming instars were found and the waters were obviously rich in H_2S. G_1 reached C 5 in late May–early June, followed by C 5 of G_2, G_3, and G_4 in July, August, and September–October, respectively, adults of each generation appearing shortly afterward. G_4 presumably produced resting eggs.

Possibly some adult females of G_4 produced subitaneous eggs that hatched into a fifth generation represented by the long flat pulses of nauplii and copepodites stretching through October and November of 1970 and 1971. Unfortunately the flooding in the autumn of 1972 destroyed the pond during a year when the third and fourth generations were early and the production of a fifth generation was most probable.

The four annual generations of *D. reighardi* in Black Pond were more than is normally found among diaptomid copepods although Comita (1972) has shown five for *D. siciloides* in Severson Lake, Minn. *D. oregonensis* in Sunfish Lake, Ont. has either two or three annual generations depending upon trophic conditions (Lai and Carter 1970) while Rigler's unpublished data suggests it has three generations

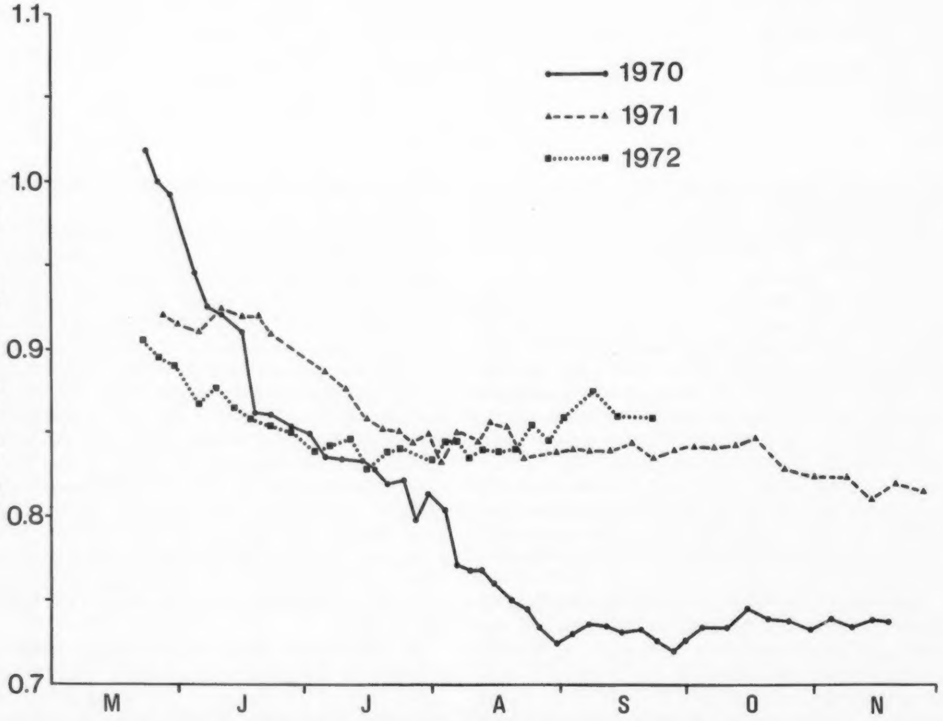

FIG. 6. Mean cephalothorax lengths of *D. reighardi* females in Black Pond, 1970–72.

in Teapot Lake, Ont. In Saskatchewan ponds Sawchyn and Hammer (1968) found that *D. forbesi*, *D. leptotus*, and *D. nudus* were multicyclic but none was shown to have more than three annual generations. Undoubtedly *D. reighardi's* unique position as the sole diaptomid in Black Pond contributed to its success since a congeneric competitor might have necessitated a division of the total time available in the plankton.

Time intervals between successive instar medians (Table 2) were derived by the same method as for *M. edax*; however, the margin of error was obviously greater for *D. reighardi* since on four occasions the estimates showed apparent negative growth (Table 2). The final column suggests that instar duration increased somewhat with age: the mean instar interval from N 1 through N 6 was about 2 days or slightly less; from N 6 through C 4, about 2.5–3 days; and from C 4 to C 5, 3.6 days. The lower two rows suggest that generation length varied both seasonally and annually. G_2 and G_3 in 1970 and 1971 developed from N 1 through C 5 in approximately 3 wk. Another 5–7 days being allowed for the interval C 5–C 6, as indicated by the data in Fig. 5, and another 2–3 days for egg development at summer temperatures (Table 3), the entire cycles from egg to adult were completed in about 30 days. G_4 in 1970 and 1971 developed mainly in the cooler waters of autumn and each required about 35 days between N 1 and C 5; the complete cycles probably took at least 1½ mo to accomplish. In 1972 G_1 reached C 5 in 20 days and probably completed the cycle in about a month. G_2 and G_3 in 1972 were almost impossible to separate in their earlier stages and likely the calculated N 1–C 5 duration is too high in the case of G_2 (26.5 days) and too low in the case of G_3 (9.1 days); since their C 1–C 5 durations were roughly equal it is likely that G_2 had a typical 1-mo summer development period whereas that of G_3 was probably somewhat shorter. G_4 in 1972, developing under summer temperatures, took only 23 days from N 1 to C 5 and may be supposed also to have had a 1-mo development period.

The most striking feature of *D. reighardi's* life history over the 3-yr period was the accelerated development rates of the latter part of 1972. This was probably due, as in the similar case of *M. edax*, to the lowered water levels increasing the pond's mean heat content per unit volume. G_3 in 1972 reached C 5 almost 3 wk earlier than in 1970 or 1971 whereas G_4 reached C 5 1 mo earlier. The same cause may have been responsible in part for the unusually large numbers of G_1 in 1972 (Fig. 5). With more heat reaching the bottom in early spring possibly a larger number of resting eggs were stimulated to hatch at once, thus enlarging the size of the population. Another reason for the high population densities of this generation, as well as of nauplii of G_2–G_4 at most times from late May through August, was that, with the reduced depth of the pond in 1972, the population was contained within a smaller volume of water.

Discussion

Though *M. edax* and *D. reighardi* coexisted from late spring to early autumn and had similar numbers and durations of generations, their development patterns reflected behavioral differences that sug-

TABLE 3. Mean times (hours) to 50% hatch in laboratory for eggs of *D. reighardi* and *M. edax*.

	D. reighardi		*M. edax*	
Temp (*C*)	Time	No. of egg sacs	Time	No. of egg sacs
5.0	no eggs formed	30	none hatched in 1000 h	10
10.0	218.2±9.1[a]	11	307.1±5.3	10
13.0	113.4±1.6	14		
14.0			106.9±0.8	13
17.0			79.2±0.4	12
18.0	74.3±1.1	11	71.8±0.7	13
21.0			52.6±0.8	13
23.0	43.7±0.7	29		
31.0	27.7±0.4	13	29.6±0.4	15

[a] One standard deviation of mean.

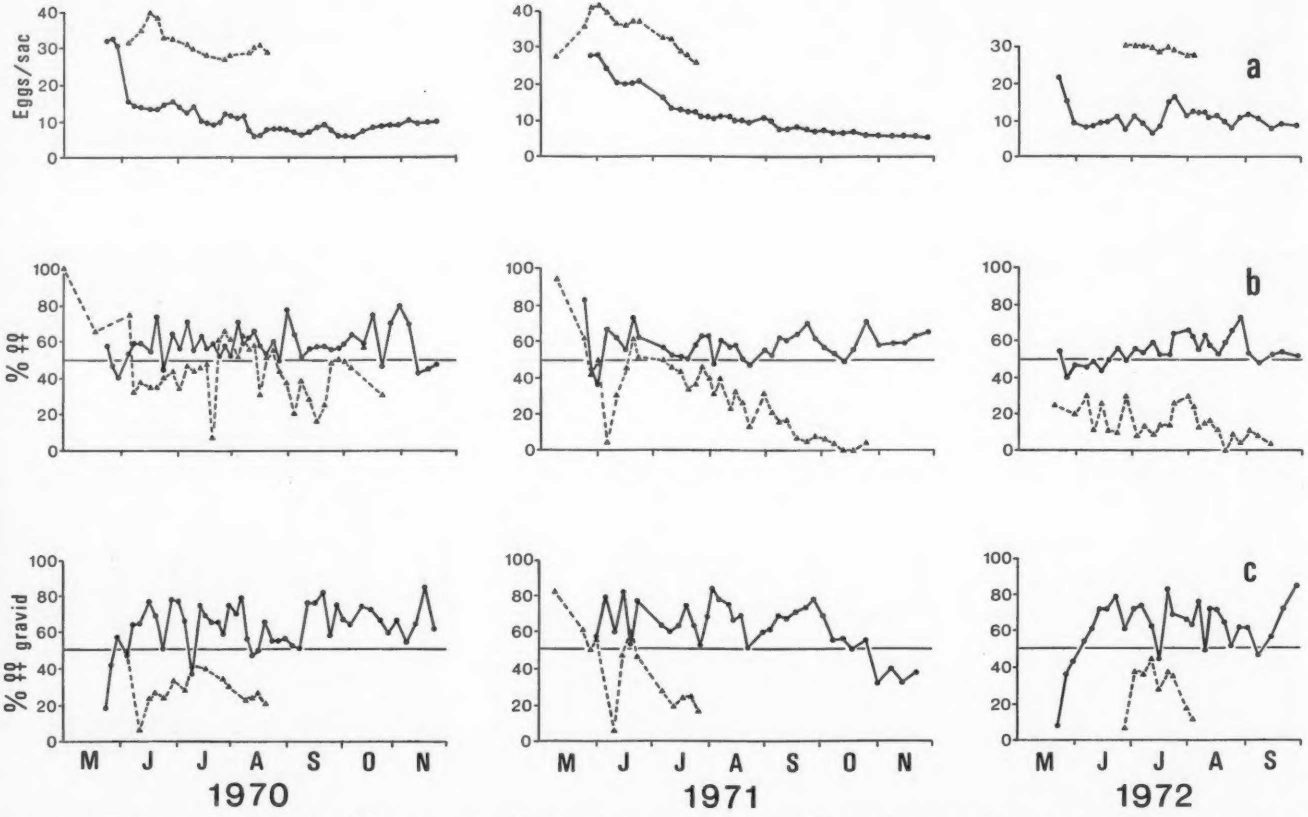

FIG. 7. Three factors in reproduction of *D. reighardi* (solid lines) and *M. edax* (broken lines) in Black Pond, 1970–72: mean numbers of eggs per egg sac (single sacs for *D. reighardi* and double sacs for *M. edax*), percentages of adults female, and percentages of females carrying eggs.

gest both a complex interrelationship and a certain ecological separation.

Usually the relationship of cyclopoid to calanoid copepods has been considered that of a simple predator–prey interaction. There is little doubt that in the adult stages most large cyclopoids are carnivorous. In laboratory feeding experiments, Confer (1971) found that adult *M. edax* prey heavily upon *Diaptomus* copepodites. If *M. edax* is a predator then it must have represented the apex of the trophic pyramid in Black Pond, since neither fish nor predaceous cladocerans were present, and should therefore be expected to have had a lower fecundity than its prey. However, except for a brief period during early spring of each year gravid *M. edax* females carried, on average, about double the number of eggs carried by *D. reighardi* females (Fig. 7a) but the egg development times of the two species were roughly equivalent (Table 3). This higher fecundity of *M. edax* females might have merely served to offset its considerably lower female: male ratios (Fig. 7b) and percentages of females carrying eggs (Fig. 7c) on almost all occasions. On the other hand it is questionable whether *M. edax* females and eggs were adequately sampled in the plankton. Both Moore (1939) and Cole (1955) suggest that adult females are mainly benthic during extended periods. As there is no additional evidence that *M. edax* either produces fewer females than males or that certain females are sterile, it is likely that many gravid females in Black Pond were usually benthic. On this basis, *M. edax* did have higher rates of both fertility and mortality than *D. reighardi* and, since survivorship of copepodites and adults did not differ greatly between the two (Fig. 4 and 5), the higher mortality must have occurred in egg or naupliar stages.

At least three explanations might account for the high losses in the early stages of *M. edax*: 1) reduced egg viability, for which no evidence exists at present, 2) cannibalism by later instars and, 3) intense competition at the naupliar level from *D. reighardi*. Cannibalism among cyclopoids has been suggested by Hutchinson (1967) and might account for situations where a cyclopoid may be the only zooplankter present. With respect to competition it is still uncertain what the nauplii of any copepod feed upon in nature although it is probably dissolved organic material, fine suspended matter, or minute organisms such as μ flagellates or bacteria. However, in the Georgian Bay area there is some suggestion that *D. reighardi* may have a depressing effect on *M. edax* populations: in a small water body similar to Black Pond and 25 km distant (Pond 11 in Carter 1971) no species of calanoid copepod occurs, yet numbers of *M. edax* were found to be consistently higher than in any other pond of the region, all of which contain large numbers of *D. reighardi.*

In 1970 and 1971 large numbers of *M. edax* eggs were collected in the plankton whereas in 1972 comparatively few were found (Fig. 4). If most gravid females in 1972 were benthic then egg development must have been slowed somewhat by the cooler ambient water at the bottom, which might explain the delay in the appearance of the third and fourth generations noted earlier. Transference of eggs from the plankton to the bottom might provide a mechanism to delay hatching of nauplii during a period when the density of a competitor is excessively high, as it was in Black Pond in 1972.

Acknowledgments

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Migration of Anadromous Dolly Varden (*Salvelinus malma*) in Southeastern Alaska

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ARMSTRONG, R. H. 1974. Migration of anadromous Dolly Varden (*Salvelinus malma*) in southeastern Alaska. J. Fish. Res. Board Can. 31: 435–444.

Of anadromous Dolly Varden (*Salvelinus malma*) migrating from the sea into Hood Bay Creek, Admiralty Island, and two other nonlake streams nearby, 61–87% were immature. Most of the immature fish left the streams within a few days of entering. Though nearly all smolts leaving the creek for 3 yr were marked, only 16–21% of the immature fish entering the stream bore these marks. Many of the fish marked in the creek as smolt were recovered entering the two other study streams as immature fish but not as spawners.

Mature immigrants remained in the streams for several weeks until they had completed spawning in October and November. Most Dolly Varden that spawned in the creek had been marked there previously as smolt or as spawners, and none were observed to spawn in a stream other than the stream of origin. Smolts transplanted during their oceanward migration returned to spawn in the receiving stream, and adults displaced to saltwater sites on returning to the study streams returned to their home streams. Most fish from the creek spawned only once or twice. Males suffered a much higher spawning mortality than did females.

This study and others on fish from lake-stream systems suggest that (1) anadromous Dolly Varden from nonlake streams in southeastern Alaska migrate both between nonlake streams and between nonlake streams and lake-stream systems; (2) during their first summer in the sea, fish from nonlake streams enter several streams while searching for one with a lake; (3) mature fish from nonlake streams leave their wintering area, usually a lake, and return to their home streams to spawn; and (4) mature fish from lake-stream systems go to the ocean to feed for several weeks to a few months and return to their home system to spawn and overwinter.

ARMSTRONG, R. H. 1974. Migration of anadromous Dolly Varden (*Salvelinus malma*) in southeastern Alaska. J. Fish. Res. Board Can. 31: 435–444.

Parmi les Dolly Vardens (*Salvelinus malma*) qui émigrent de la mer vers le ruisseau Hood Bay (île de l'Amirauté) et vers deux autres cours d'eau avoisinants non reliés à un lac, 61–87% sont immatures. La plupart des poissons immatures quittent les cours d'eau après y être restés seulement quelques jours. Bien que presque tous les smolts qui ont quitté le ruisseau au cours d'une période de 3 ans aient été marqués, seulement 16–21% des poissons immatures pénétrant dans le cours d'eau portent de ces marques. Plusieurs des poissons marqués dans le ruisseau comme smolts sont recapturés au moment où ils pénètrent dans les deux cours d'eau sous étude, comme immatures et non comme reproducteurs.

Les immigrants mûrs demeurent plusieurs semaines dans les cours d'eau, jusqu'à la fin de la fraie en octobre et novembre. La plupart des Dolly Vardens qui fraient dans le ruisseau ont été marquées auparavant au stade de smolts ou comme reproducteurs, et on n'en a vu aucune frayer dans un cours d'eau autre que celui d'origine. Des smolts transplantés lors de leur migration vers l'océan sont retournés dans le cours d'eau qui les avait reçus, et des adultes déplacés vers des sites en eau salée au moment de leur retour vers les cours d'eau sous étude sont retournés à leur cours d'eau d'origine. La plupart des poissons provenant du ruisseau ne fraient qu'une ou deux fois. Les mâles subissent des mortalités beaucoup plus élevées que les femelles lors de la fraie.

Cette étude, ainsi que d'autres effectuées sur des poissons provenant de régimes lac-cours d'eau suggèrent que (1) les Dolly Vardens anadromes de cours d'eau non reliés à un lac dans le sud-est de l'Alaska se déplacent à la fois entre les cours d'eau non reliés à un lac et entre ceux-ci et des régimes lac-cours d'eau; (2) durant leur premier été en mer, les

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poissons provenant de cours d'eau non reliés à un lac pénètrent dans plusieurs cours d'eau, à la recherche d'un qui possède un lac; (3) les poissons mûrs provenant de cours d'eau reliés à un lac quittent les lieux d'hivernage, ordinairement un lac, et reviennent à leur cours d'eau d'origine pour frayer; et (4) les poissons mûrs provenant de régimes lac-cours d'eau se rendent en mer pour se nourrir pendant une période allant de plusieurs semaines à quelques mois, et reviennent à leur régime natal pour frayer et y hiverner.

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The migratory habits of anadromous Dolly Varden (*Salvelinus malma*) are unusual and possibly unique. They leave lakes in the spring and early summer, migrate in many directions at sea, enter nonlake streams, and return to the lakes before winter (Armstrong 1965). Some of the fish entering lakes have spawned recently in other stream systems, others will spawn later in the year in streams connected to the lake, and still others are immature fish not going to spawn that year (Blackett 1968). Most of the immature fish have not been in the lake system before (Armstrong 1965; Heiser 1966). Although these points have been well documented, many details are still uncertain. Of special importance to fisheries managers is the question of homing — do Dolly Varden have home streams for spawning and development to the smolt stage and do nonspawning fish enter their home streams? A search of the literature (Armstrong and Morton 1969) revealed no studies on homing of anadromous Dolly Varden to their natal streams.

In this report I present the results of a study of anadromous Dolly Varden from three nonlake streams on Admiralty Island in southeastern Alaska. I examine population numbers and biological and behavioral characteristics of marked fish in the streams to determine migration habits of both nonspawners and spawners. The results of the study are related to earlier studies concerning a stream with a lake to develop a general hypothesis of migration for Dolly Varden.

Materials and Methods

Migrating Dolly Varden were counted, sampled, and marked as they passed upstream and downstream at weirs built across Hood Bay Creek in the South Arm and two streams entering the North Arm of Hood Bay (Fig. 1). The weirs (Armstrong and Winslow 1968; Armstrong and Kissner 1969) were capable of trapping all sizes of migrating Dolly Varden. The streams are in a pristine area, and the Dolly Varden are fished very little.

All Dolly Varden captured at the Hood Bay Creek weir in 1966–69 and at the weirs on the North Arm streams in 1968 were counted and measured (fork length). In addition, in 1967–69, every 10th upstream migrant was killed to permit determination of age, sex, and stage of gonadal development; in 1966, every 20th upstream migrant was killed. Representativeness of the sample killed at each weir was tested by comparing the length frequencies of the fish sampled and those measured and counted only upstream. Chi-square tests showed that, for 1967 and 1969 at Hood Bay Creek and for 1968 at the North Arm streams, there were no significant differences ($\alpha = 0.05$) between the length frequencies of the two groups. For 1968 at Hood Bay Creek, the difference was significant because of fewer small fish (100–170 mm) and more large fish (230–280 mm) in the samples than the counts. However, the percentages of spawners in the samples (22%) and the counts (23%) were nearly identical. For 1966 at Hood Bay Creek the sampling is considered to be only a reasonable approximation of the maturity composition of the immigrants.

The total numbers that entered the streams were estimated from ratios of marked to unmarked fish (Table 1). All fish captured and released as they passed upstream were marked by punching a hole in the

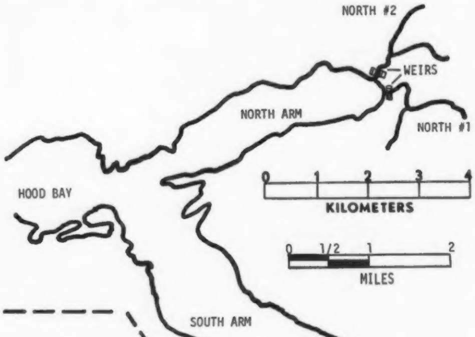

Fig. 1. Study area, Admiralty Island, southeastern Alaska.

caudal fin with a paper punch. As all Dolly Varden capable of leaving the nonlake streams do leave the same year they enter, the estimates were based on the marked-to-unmarked ratios of the emigrants. The estimated numbers missed were proportioned over periods when the weir screens were removed to accommodate flood waters. The estimates were based on the numbers captured before and after screen removal.

Age was determined from the number of hyaline rings (annuli) formed on otoliths (Heiser 1966) removed from the sampled fish.

The degree of maturity of each fish sampled was determined by dissection and visual examination of the gonads (Blackett 1968). Whether or not the fish had recently spawned was determined from their external characteristics (Blackett 1968).

The stream from which the smolt originated or in which spent adults had spawned was recorded on each fish by marking the fish as they passed the weirs on their way to the ocean. At the Hood Bay Creek weir in 1967, 1968 and 1969 smolt were marked by removing the right, left, or both ventral fins, and spent fish in 1966–68 by removing the adipose fin and tagging with dart tags (Armstrong and Blackett 1966). Spent adults captured at the North Arm streams in 1968 were marked with internal anchor tags (Dell 1968). In 1966 and 1967 all fish were tagged with numbered dart tags as they entered the creek and recorded when recaptured at the weir as they left. All spent Dolly Varden captured while leaving the three study streams in 1969 were examined for finclips and tags.

An experiment was conducted to determine if the smolt could be imprinted for homing by brief exposure to a recipient stream at the time of oceanward migration. Smolt were captured at the weirs on the North Arm streams in May and June 1969 and transported to Hood Bay Creek, where they were measured, finclipped, held in the creek for 24 h, and released below the weir. Whether imprinting occurred was determined by comparing the percentages of returnees from the transplanted fish with those from smolts native to the creek.

To further test the homing abilities of Dolly Varden, fish were captured at the weirs as they returned to fresh from salt water, marked, and displaced to various

TABLE 1. Estimates of numbers of Dolly Varden (*Salvelinus malma*) entering the Hood Bay streams, 1966–69.

	Immigrants marked	Emigrants		Estimate of immigrants[a]
		Unmarked	Marked	
Hood Bay Creek				
1966	2843	429	552	5533
1967	2894	1389	1794	5954
1968	2275	664	639	5007
1969	3889	1540	1734	7904
North Arm streams (1968)				
No. 1	2759	230	124	8285
No. 2	1997	105	95	4653

[a] Includes numbers of fish dead in traps and those used in displacement experiments as well as those sampled.

TABLE 2. Estimated percentages of Dolly Varden entering the three study streams that were nonspawners, and of spawners that were females, 1966–69.

	Estimated total	Nonspawners (%)	Spawners		No. sampled
			Total	Females(%)	
Hood Bay Creek					
1966	5533	61	2158	46	131
1967	5954	70	1799	62	338
1968	5007	71	1448	77	269
1969	7904	80	1608	64	462
North Arm streams (1968)					
No. 1	8285	87	1045	51	311
No. 2	4653	84	738	59	243

saltwater sites where they again had to choose which stream to enter. In 1967 and 1968, 565 fish were displaced from the three study streams up to 9.5 km from the streams, in 1967, 286 fish from the creek, and in 1968, 279 fish — 76–102 from each of the three study streams — to the junction of the North and South arms. Recoveries were made at the weirs.

Results

Dolly Varden of two distinct stages of sexual maturity migrated into the streams of Hood Bay between July and October each year: mature fish going to spawn that year (spawners) and immature fish not going to spawn that year (nonspawners). Between 13 and 39% of the migrants into the streams each year were spawners (Table 2). The major period of stream entry was August and September for spawners and September for nonspawners (Fig. 2). Dolly Varden that entered Hood Bay Creek in the summer and fall apparently either returned to sea or died before December; many fish which had recently entered left (Fig. 3) between July and mid-November. Intensive visual searches failed to find any fish of migratory size in the entire system after mid-November.

Most nonspawners left the creek within a few days of entering, whereas most spawners remained for several weeks, until they had completed spawning. In 1966, nonspawners returning to sea (*n*=121) had spent an average of 14 days above the weir and 75% of them left within 10 days; spawners returning to sea (*n*=261) had spent an average of 83 days above the weir. In 1967, nonspawners (*n*=527) had spent an average of 14 days above the weir and 58% left within 10 days; recaptured spawners (*n*=420) had spent an average of 72 days above the weir (Fig. 3).

In 1967 more than 80% of the nonspawners had left the creek before the spent fish began leaving (Fig. 4). In both years, all the spent fish left in the 1-mo period from mid-October to mid-November; the females (523 tagged fish recaptured) began leaving before the males (158 recaptured) and spent less time (74 vs. 83 days) in the creek.

More than 90% of the fish entering the study streams were of ages II–VI, and the rest of ages VII–XI (Table 3). More than 90% of the total number of spawners were of ages IV–VII, and more than 90% of the total number of nonspawners of ages II–V. No age II fish were mature and more males than females were mature at ages III and IV.

The percentage age distributions of 200 female and 109 male Dolly Varden spawners sampled as they entered Hood Bay Creek were:

Age	♀	♂	Total
III	0.5	10.1	3.9
IV	16.5	30.3	21.4
V	41.0	22.9	34.6
VI	25.0	19.3	23.0
VII	12.0	11.0	11.6
VIII	4.5	5.5	4.9
IX	0.5	0.9	0.6

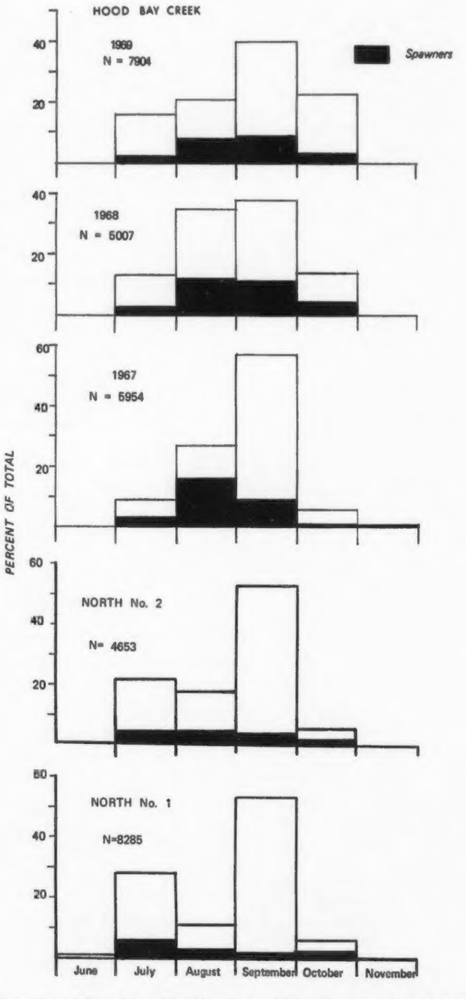

FIG. 2. Monthly distributions (% of annual totals) of spawner and nonspawner Dolly Varden (*Salvelinus malma*) entering Hood Bay Creek, 1967–69, and the two North Arm streams in 1968.

Length frequency distributions were similar for the fish entering and leaving Hood Bay Creek. The length ranged from about 91 to 540 mm (Fig. 5) — most of the nonspawners were less than 280 mm and most of the spawners were over 320 mm.

MIGRATION OF NONSPAWNERS

Nonspawning fish migrated from one nonlake stream to another. Most of the nonspawners entering the creek originated elsewhere. The percentages of the estimated numbers of nonspawners entering the creek each year that had been marked as smolts leaving the creek were:

	1967	1968	1969
Total nonspawning immigrants	4155	3559	6296
Hood Bay Creek origin (%)	16	21	16

The percentage age distribution of 271 of the nonspawners of unknown origin that entered the creek was: II, 16.6; III, 60.9; IV, 19.6; V, 2.6; and VI, 0.4. This was also the approximate age distribution of Dolly Varden smolt that left the creek in 1967–69 (Armstrong 1970). However, since most smolt were marked as they left in 1967–69, most of the unmarked nonspawners originated elsewhere.

Nonspawners commonly entered streams other than their natal streams. Of 5847 smolts marked in 1967 leaving the creek, 162 were recovered as nonspawners in 1968 — 94% in North Arm streams and only 6% in the creek. Of 5819 smolts marked in 1968 leaving the creek, 1218 were recovered as nonspawners in 1968 — 55% in the North Arm streams and 45% in Hood Bay Creek.

MIGRATION OF SPAWNERS

Homing — Most of the fish that spawned in the creek in 1969 had apparently returned to this stream at least once. Of the 882 spent fish captured leaving the creek in 1969, 44% had been marked as smolt emigrating from the creek in 1967–68, 31% had been marked after spawning in the creek before, and 25% were unmarked. The unmarked spawners in 1969 could have been from the 1926 smolt estimated to have migrated while the weir was out in 1968: a 33-fish sample of the unmarked spawners were of ages III (3.0%), IV (45.4%), V (39.4%), and VI (12.1%) and the smolt that migrated in 1968 (Armstrong 1970) were of ages II (3.5%), III (59.9%), IV (34.0%), and V (2.4%). Also, the fish which had been marked after spawning in the creek before may have originated from the creek because fish up to 8 yr of age were found among the spawners and none were marked before 1967.

No fish of Hood Bay Creek origin were known to spawn in either of the two North Arm streams. Though many smolt were marked while leaving the creek — 18,165 in 3 yr — none were found among the 572 spent fish captured at the weirs on the North Arm streams in 1968 and 1969.

No Dolly Varden were known to spawn in more than one of the study streams. None tagged as spent

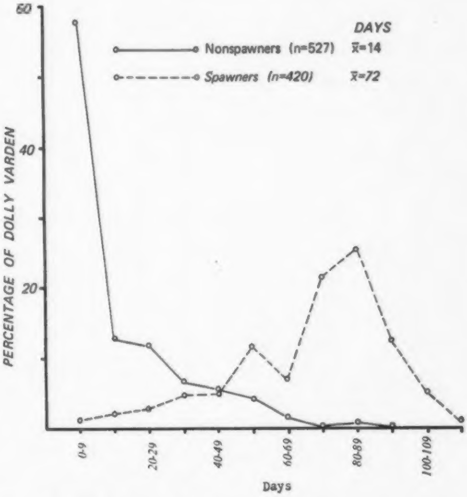

FIG. 3. Numbers of days that spawning and nonspawning tagged Dolly Varden spent in Hood Bay Creek, 1967.

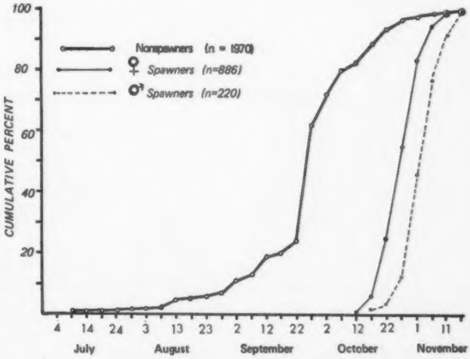

FIG. 4. Cumulative percentages of nonspawners and spent female and male Dolly Varden leaving Hood Bay Creek, 1967.

TABLE 3. Percentage age compositions of immigrant Dolly Varden spawners and nonspawners sampled at the Hood Bay Creek weir, 1967–69, and North Arm weirs, 1968.

Age-group	Hood Bay Creek									North Arm #1			North Arm #2		
	Spawners			Nonspawners			Total								
	1967	1968	1969	1967	1968	1969	1967	1968	1969	S	N	T	S	N	T
II	0.0	0.0	0.0	2.0	0.0	17.0	1.3	0.0	13.3	0.0	0.0	0.0	0.0	3.1	2.6
III	3.1	4.4	4.0	53.0	41.4	58.0	36.7	31.1	46.2	0.0	18.9	14.8	2.5	25.7	21.6
IV	11.5	17.6	41.6	30.8	46.2	21.7	24.4	39.0	26.0	28.1	59.7	52.9	25.0	47.6	43.8
V	30.2	39.7	37.6	9.6	9.1	3.0	16.3	17.3	10.5	40.6	18.0	22.9	27.5	20.4	21.6
VI	32.3	17.6	12.9	2.5	2.2	0.3	12.2	5.9	3.0	23.4	3.4	7.7	35.0	2.6	8.2
VII	14.6	11.8	3.0	1.0	0.5	0.0	5.4	3.9	0.6	7.8	0.0	1.7	7.5	0.5	1.7
VIII	7.3	7.4	1.0	1.0	0.5	0.0	3.4	2.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0
IX	1.0	1.5	0.0	0.0	0.0	0.0	0.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
XI	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.5
Sample size	96	68	101	198	186	364	294	254	465	64	233	297	40	191	231

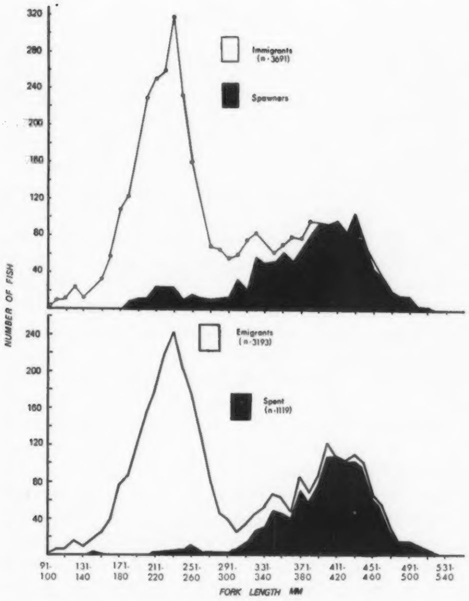

FIG. 5. Length frequency distributions of Dolly Varden passing the Hood Bay Creek weir, July–November, 1967. The numbers of immigrant spawners are estimates from a sample of immigrants (338 fish) and those of emigrant spawners are counts of spent fish.

fish in the creek from 1966 to 1968 (n=2593) were recaptured as spent fish in the North Arm streams in 1968 or 1969. Similarly, none tagged as spent fish in either of the North Arm streams in 1968 were recaptured in either of the other study streams in 1969. As spawning in consecutive years is common in Hood Bay Creek Dolly Varden, fish that spawned in more than one stream were likely to be detected.

Smolt transplant — Of 1184 finclipped smolts transplanted in May and June 1969 from the North Arm streams to the creek, the portion that returned to the creek in 1970 to spawn (10.5%) was nearly identical to the return from 5011 smolts native to the creek (9.4%). Only four spawners captured at the weirs on the North Arm streams in 1970 were from smolt transplanted to the creek.

As the transplanted smolt were held in the creek for approximately 24 h before release, these results indicate that imprinting for homing in Dolly Varden occurs sometime during the last day of smolt migration. Most of these smolts had spent 3 yr or more in the North Arm streams. The percentage age composition of a sample of 60 of the smolt at the time of transplant was: II, 1.9; III, 24.1; IV, 61.1; V, 11.1; and VI, 1.9.

Displacement experiments — Of the 565 fish displaced from fresh to salt water in 1967 and 1968, many large fish (mature) and few small ones (immature) returned the same year to the stream from which they were displaced. Of the 286 fish displaced from the creek in 1967, 23% were recaptured at the creek weir. The percentages that returned were much higher for large than for small fish:

Area of release	Km from creek weir	Size (*mm*)	No. displaced	% returned
Junction of arms	5.0	≥301	19	47.4
		≤300	81	6.2
Mouths of North Arm streams	9.5	≥301	40	42.5
		≤300	58	5.2
Mouth of Hood Bay Creek	0.5	≥301	45	53.3
		≤300	43	16.3
All areas		≥301	104	48.1
		≤300	182	8.2
Total			286	22.7

All but eight of the returned fish were ripening or were recaptured going back to salt water after spawning in the creek.

Of the 279 fish displaced from the three study streams to the junction of the North and South arms in 1968, nearly all of those recaptured at the creek weir were large (ripe or spent) fish from the creek and very few were from the North Arm streams:

Area of initial capture	Length (*mm*)	No. displaced	% recovered at creek weir
Hood Bay Creek	≥325	56	67.9
	≤275	46	0.0
North Arm Stream #1	≥325	43	0.0
	≤275	33	3.0
North Arm Stream #2	≥325	49	6.1
	≤275	52	1.9

The actual return to the creek was probably higher than that observed because some of the returned fish probably died soon after spawning and were not recovered. Only a few of the fish displaced from the North Arm streams were recovered at the creek and none spawned there.

SPAWNING FREQUENCY

Of fish from the creek, most spawned only once or twice — only occasionally were fish found that had spawned more than twice. Of 5847 smolt that were finclipped and released at the creek weir in May and June 1967, 428 were known to have spawned in subsequent years — 73% spawned once, 26% twice, and 1% three times. Only a few (nine fish) returned and spawned the year they entered the ocean as smolt. Most (69%) spawned for the first time 1 yr later and the balance (31%) 2 yr later.

However, of fish from the three study streams, most spawned each year after reaching maturity. Of 94 tagged fish recaptured in 2 or more years after they were first marked as spawners, 78% spawned either 3 or 4 yr consecutively. The remaining 22% were not recaptured each year but may have been missed at the weirs because of high waters. Hence, nonconsecutive spawning could not be established for the fish from the creek. However, this is not a significant question for Dolly Varden of this stream because only a few live more than 2 yr after emigrating as smolt.

Males had much higher mortality associated with spawning than did females. On the basis of the weir counts of mature fish entering and leaving the creek in 1967 and 1968, the mortalities (%) were:

	No. in	No. out	Mortality
	1967		
Females	937	897	4.3
Males	575	222	61.4
Total	1512	1119	26.0
	1968		
Females	1007	918	8.8
Males	297	151	49.2
Total	1304	1069	18.0

The higher spawning mortality of males was reflected in a greater abundance of females than males among spent fish leaving the creek. This was especially evident in those fish spawning more than once — no males survived to spawn more than twice. The percentage of females and males among spent fish captured at the creek weir after various numbers of spawnings were:

No. of spawnings	1	2	3	4
% female	75	89	100	100
Sample size	472	607	68	5

Discussion

On the basis of the results of this study on Dolly Varden in streams without lakes and of others for streams with lakes (Armstrong 1965; Blackett 1968; Heiser 1966), I believe the migration habits of anadromous Dolly Varden in southeastern Alaska are as follows:

Fish that hatch and develop in nonlake streams enter the sea as smolts in spring and fall, primarily at ages II–IV. At sea, these fish search for a stream with a lake wintering area,[1] and in this search, they may enter and leave several streams. Sometime between July and November the fish enter a lake-stream system and remain there until the following spring, spending the winter in the lake. In spring, they return to sea and, if they are still immature, may make excursions into streams other than thier home stream before they return to the lake-stream system from July through November. At maturity, after migrating to sea from their wintering area in March through June, they enter their home stream from July through October to spawn in September through November. The survivors of spawning return to sea and again enter a lake-stream system to overwinter. Most mature fish probably do not enter other streams between their lake wintering area and home stream. Also, spent fish migrating from nonlake streams to their overwintering lakes probably do not enter other streams.

Fish that develop in a lake-stream system enter the ocean as age III and IV smolt in May and June. Most probably they do not enter other streams but return to their home lake-stream system before winter. Both immature and mature fish presumably migrate from the system to the ocean and back again annually after transforming to smolt. Fish that spawn in tributaries to a lake probably return to the lake to spend the winter.

OTHER AREAS OF ALASKA

Only cursory studies have been made of the migrations of anadromous Dolly Varden elsewhere in Alaska but the results do not disagree with the migration patterns described here for southeastern Alaska.

The fish are known to winter in lakes or large rivers in several areas in Alaska. These include the Ugashik Lakes in Bristol Bay (Baade 1955a); Chignik Lake on the Alaska Peninsula (Roos 1959); Bear Lake near Seward (Logan 1962); Karluk Lake

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[1] Although not common, there are some larger rivers in southeastern Alaska in which Dolly Varden overwinter. However, for ease of discussion, the wintering areas will be referred to as lakes.

(DeLacy and Morton 1942), Bare Lake (Nelson 1959), and Buskin Lake (Marriott 1967) on Kodiak Island; Kitoi Lake on Afognak Island (Meehan 1966; Revet 1962); the Chilkat River near Haines (Baade 1955b); and several lakes in southeastern Alaska (Baade 1957; Chipperfield 1937, 1938).

Migrations between nonlake and lake systems probably occur elsewhere in Alaska. After September 29, nearly all the Dolly Varden captured moving upstream at the Bear Lake weir had spawned prior to reaching the weir (Logan 1962), indicating that they had spawned in another system. Dolly Varden tagged in the American River, a nonlake stream on Kodiak Island, have been captured leaving Buskin Lake (Marriott 1968). Earlier Marriott (1965) had observed: "Despite a population of several thousand Dollies observed in the upper Buskin and later in tributaries above Buskin Lake, the number actually spawning is very small, and probably consists of less than 500 fish." Dolly Varden entering nonlake streams and retreating to sea before winter have been reported for Big Kitoi Creek on Afognak Island and Ketchikan Creek in southeastern Alaska (Rounsefell 1958).

Anadromous Arctic char (*Salvelinus alpinus*) in Alaska may make migrations similar to those of Dolly Varden. A population of about 200,000 Arctic char has been estimated for the Wulik River, a large river system, near the Eskimo village of Kivalina (Winslow 1969a). These fish leave this system in June and return in August and September (Saario and Kessel 1966). The people of Kivalina conduct a substantial winter fishery on these fish in the Wulik River (Winslow 1969a). Large numbers of Arctic char spend the winter in Wulik River, migrate to the ocean in spring, and return in late summer, but apparently many are from other systems. An intensive autumn fishery takes many fish, but char in spawning condition are seldom caught and char younger than age III are seldom found (Winslow 1969b). Winslow considered that relatively few char spawned in the Wulik and that segments of the autumn–winter population may have originated from other systems. Also, the Eskimos at Kivalina have mentioned that large red char (probably spent fish) move into the system under the ice in November and although Arctic char are found in smaller streams nearby in summer and fall, they leave before winter (P. C. Winslow personal communication).

MANAGEMENT IMPLICATIONS

Successful management of anadromous Dolly Varden requires thorough understanding of their complex migration habits. Of special importance is management of key wintering areas such as lakes and larger river systems. For 5–9 mo each year, each wintering area may harbor fish from many streams scattered over a vast area. A depletion of the fish in one wintering area could severely reduce or eliminate populations belonging to many stream systems.

Migrations between nonlake streams further complicate management of Dolly Varden. Attempts to build up a depleted run in one stream might prove futile if the fish, after stocking, moved into other heavily fished streams. The possibility that the fish which home to one stream may be found in lakes, other streams, and the ocean at various stages of their migrations must be recognized and these various bodies of water and their fish should be managed as a complex.

The relatively few spawners that enter each stream is another important consideration. After sampling Dolly Varden from approximately 50 streams on Admiralty, Baranof, and Chichagof islands in 1962 and 1963, I was particularly impressed by the numbers of spawners sampled at Hood Bay Creek and concluded that a "typical" spawning run into streams of southeastern Alaska is about 1000 fish or less. The immigrations of 1500–2000 spawners at Hood Bay Creek appear to be more than usual in most streams in southeast Alaska.

The difference in time that spawning and nonspawning Dolly Varden spend in streams also needs to be considered. Spawners stayed an average of 72–83 days in Hood Bay Creek while nonspawners stayed an average of only 14 days and more than half left the stream within 10 days. Hence, spawners would be more readily available than nonspawners to stream anglers. Creel censuses of Dolly Varden fishermen in streams should include estimates of maturity of the catch to aid in determining accurate harvest data on the spawners.

Complete protection of spent Dolly Varden may be advisable in heavily fished areas. Although mortality after spawning is high among males, up to 50% of the females spawning for the first time may survive to spawn again. These larger females make substantial contributions to reproduction of the population. Closures during October and November would protect the spent fish during their migration from nonlake streams into lakes.

In southeastern Alaska, lakes in which anadromous Dolly Varden overwinter will become prime recreation areas as human populations increase and as the land becomes developed. Industry, power projects, tourist resorts, and residential development may not be compatible with maintaining a large wintering population of fish. Some major Dolly Varden wintering areas should be preserved from development, and fishing in these areas regulated to protect the stocks involved.

Acknowledgments

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NOTES

Muscle Arylamidase Activity of Several Marine Species

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BAUER, B. A., AND R. R. EITENMILLER. 1974. Muscle arylamidase activity of several marine species. J. Fish. Res. Board Can. 31: 445–448.

Arylamidase activity in muscle extracts of mackerel, mullet, whiting, blue crab, quahog clam, and shrimp was investigated. The optimum *p*H for activity was between 7.0 and 7.5 for each species using alanyl-β-naphthylamide as substrate. Enzymes of the three species of fish exhibited maximum activity against alanyl-β-naphthylamide, whereas the clam showed maximum activity with leucyl-β-naphthylamide and the crab and shrimp with lysyl-β-naphthylamide. Puromycin inhibited each of the arylamidases. Acrylamide gel electrophoresis of the crude extracts suggested the presence of one arylamidase in muscle of the fish, crab, and clam. The shrimp muscle extract contained two active enzymes. Upon electrophoresis, the faster moving enzyme from shrimp muscle was active against lysyl-, alanyl-, and leucyl-β-naphthylamides, but the slower moving enzyme was active only against lysyl-β-naphthylamide.

BAUER, B. A., AND R. R. EITENMILLER. 1974. Muscle arylamidase activity of several marine species. J. Fish. Res. Board Can. 31: 445–448.

Nous avons étudié l'activité de l'arylamidase dans des extraits musculaires de maquereau, muge, merlu, crabe bleu, palourde et crevette. Le *p*H maximal d'activité se situe entre 7.0 et 7.5 pour chaque espèce, avec l'alanyl-β-naphthylamide comme substrat. Les enzymes des trois espèces de poisson exhibent un maximum d'activité avec l'alanyl-β-naphthylamide; par ailleurs, la palourde montre un maximum d'activité avec la leucyl-β-naphthylamide, et le crabe et la crevette avec la lysyl-β-naphthylamide. La puromycine inhibe chacune des arylamidases. L'électrophorèse sur gel acrylamide d'extraits crus suggère la présence d'une arylamidase dans le muscle des poissons, du crabe et de la palourde. L'extrait musculaire de la crevette contient deux enzymes actifs. A l'électrophorèse, l'enzyme plus rapide du muscle de crevette est actif contre les lysyl-, alanyl- et leucyl-β-naphthylamides; par ailleurs, l'enzyme moins rapide n'est actif qu'avec la lysyl-β-naphthylamide.

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ARYLAMIDASES are enzymes with the capability of hydrolyzing amino acid-β-naphthylamides. Since their discovery (Patterson 1963), arylamidases have been demonstrated to be widespread in nature. The enzymes are inhibited by puromyocin and can be differentiated from other exopeptidases such as leucine aminopeptidase by this property (Marks et al. 1968). They have been isolated and characterized from several mammalian tissues (Brecher et al. 1969; Behal et al. 1969; and Makinen and Raekallio 1968) and microbial sources (Behal and Cox 1968; Behal and Carter 1971; and Riley and Behal 1971), but the in vivo function of arylamidase has not been clarified. Studies by Ellis and Nuenke (1967) and Tappel (1968) provide evidence that arylamidase acts in degrading oligopeptides. Rustow et al. (1971) found that the arylamidase activity in rat intestine, as well as the activity of leucine aminopeptidase and tripeptidase, increased when the protein efficiency ratio of the diet was decreased, also suggesting a role in proteolysis.

As part of a larger study of enzymes involved in proteolytic breakdown of marine muscle, the presence of arylamidase in the muscle tissue of three species of fish and of three invertebrates was investigated. The fish species included whiting, Spanish mackerel, and mullet. The invertebrates were blue crab, quahog clam, and white shrimp.

Materials and methods — Enzyme assay — Arylamidase activity was assayed by the method of Goldbarg and Rutenburg (1958). One-tenth milliliter of enzyme

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solution and 0.9 ml of 0.1 M phosphate buffer, *p*H 7.0, were preincubated for 10 min at 37 C, after which 1.0 ml of prewarmed 6.85 × 10^{-4} M amino acid-β-naphthylamide in 0.1 M phosphate buffer, *p*H 7.0, was added. Reaction time varied from 15 to 120 min and all reactions were terminated before 50% substrate hydrolysis was reached. Standard curves were prepared using β-naphthylamine (Sigma) at concentrations from 10 to 100 μg/ml (0.685 × 10^{-4} to 6.85 × 10^{-4} M). Specific activity was reported as μg β-naphthylamine released/h per milligram protein. Protein was measured by the biuret reaction (Gornall et al. 1949). All enzyme assays were done in duplicate and values reported are averages of two or more trials.

Preparation of muscle extracts — The marine species were obtained fresh from docks at Brunswick, Ga., or from seafood wholesale houses in Athens, Ga. The various species were either used immediately or held frozen at –30 C until assayed. Homogenates (1:4 wt/vol) were prepared by blending excised muscle in 0.1 M phosphate buffer, *p*H 7.0, containing 4 mM mercaptoethanol for 30 s. The homogenates were centrifuged at 17,500 × *g* for 10 min at 4 C and the insoluble material was discarded. Only claw muscle from the blue crab and white muscle from the mackerel were used. Care was taken to avoid contamination of all extracts with digestive enzymes.

*p*H *optima* — Using alanyl-β-naphthylamide as substrate, optimal *p*H studies were carried out using 0.1 M phosphate buffer for *p*H values from 5.5 to 8.5 and 0.1 M Tris buffer from 8.5 to 9.5.

Substrate specificity — Substrate solutions were prepared by mixing equal amounts of 0.00137 M substrate and 0.2 M phosphate buffer, *p*H 7.0. The amino acid-β-naphthylamides used were cystine dihydrochloride, histidine, isoleucine hydrobromide, aspartate, glycine hydrochloride, lysine carbonate, tryptophan (Schwarz-Mann), and alanine and leucine hydrochloride (Sigma).

Puromycin inhibition — Inhibition by puromycin was studied by reacting the extracts from the various marine species with substrate at concentrations varying from 0.685 × 10^{-4} M to 6.85 × 10^{-4} M in the presence of the inhibitor. Puromycin concentrations varied from 0.25 μM to 2.5 μM for the fish enzymes, from 1.0 μM to 10 μM for clam arylamidase, from 0.01 μM to 0.1 μM for crab arylamidase, and from 0.001 μM to 0.1 μM for shrimp enzyme. The enzyme was preincubated with the inhibitor for 15 min at 37 C before the addition of alanyl-β-naphthylamide. The data were plotted as inverse velocity vs. the inverse of the substrate concentration, and the inhibitor constants were calculated from the change in the slope value, intercept value, or both.

Acrylamide gel electrophoresis — A modification of the method of Davis (1964) was used. Gel *p*H was 7.5 and the chamber buffer was 0.05 M phosphate, *p*H 7.0. Extract samples containing sucrose were layered on 6.0% gels and overlayered with buffer. Gels were run at 5–8 C at 3 ma/tube for 3 h. Histochemical stains of arylamidase active bands were made by incubating the gels in substrate (6.85 × 10^{-4} M) for 1 h before placing them in a 0.14% Fast Garnet (Sigma) solution in 0.2 M acetate buffer, *p*H 4.5, for 1 h. Active bands appeared as brick-red bands against a clear background. Gels were destained and stored in 7% acetic acid.

Results and discussion — *p*H *optima* — The optimum *p*H for the muscle arylamidase activity was between 7.0 and 7.5 for each species examined, using alanyl-β-naphthylamide as substrate. The *p*H optima were broad with high activity present, one *p*H unit on both sides of the optimal *p*H. These *p*H optima indicate that the arylamidases would probably be found in the muscle at or near their optimal *p*H values.

The optimal *p*H values found in this study agree with optima found for arylamidases from other sources. Using alanyl-β-naphthylamide as substrate, brain arylamidase had a *p*H optimum of 7.5 (Brecher and Suszkiw 1969) as did arylamidase isolated from *Sarcina lutea* (Behal and Carter 1971). Dipeptidyl, arylamidase II of the pituitary gland had an optimal *p*H of 7.0–7.5 (McDonald et al. 1968).

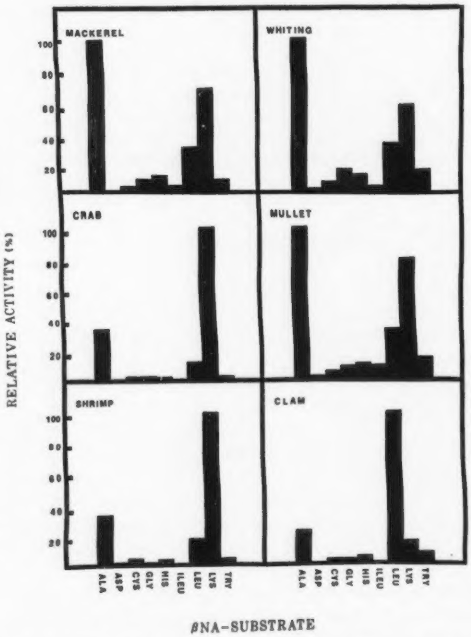

Fig. 1. Substrate specificity profiles of the six marine muscle arylamidases.

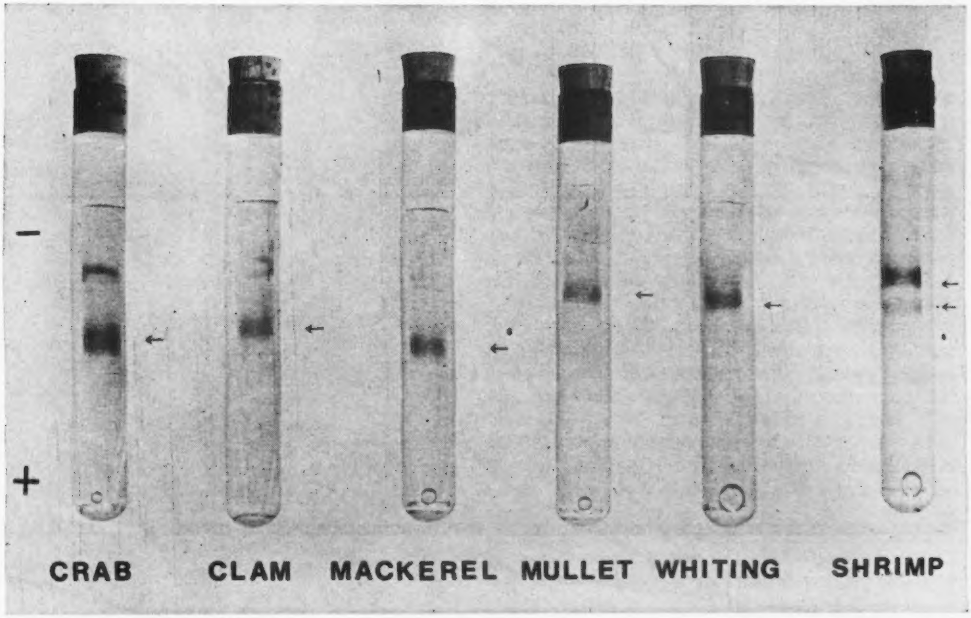

FIG. 2. Acrylamide gels stained histochemically for activity after incubation in lysyl-β-naphthylamide. The upper band present in the crab gel was not due to enzymatic hydrolysis of the arylamide substrate, but to nonspecific reaction of a component in the extract with a component of the histochemical stain. The band was not fluorescent before staining or characteristic in color after histochemical staining.

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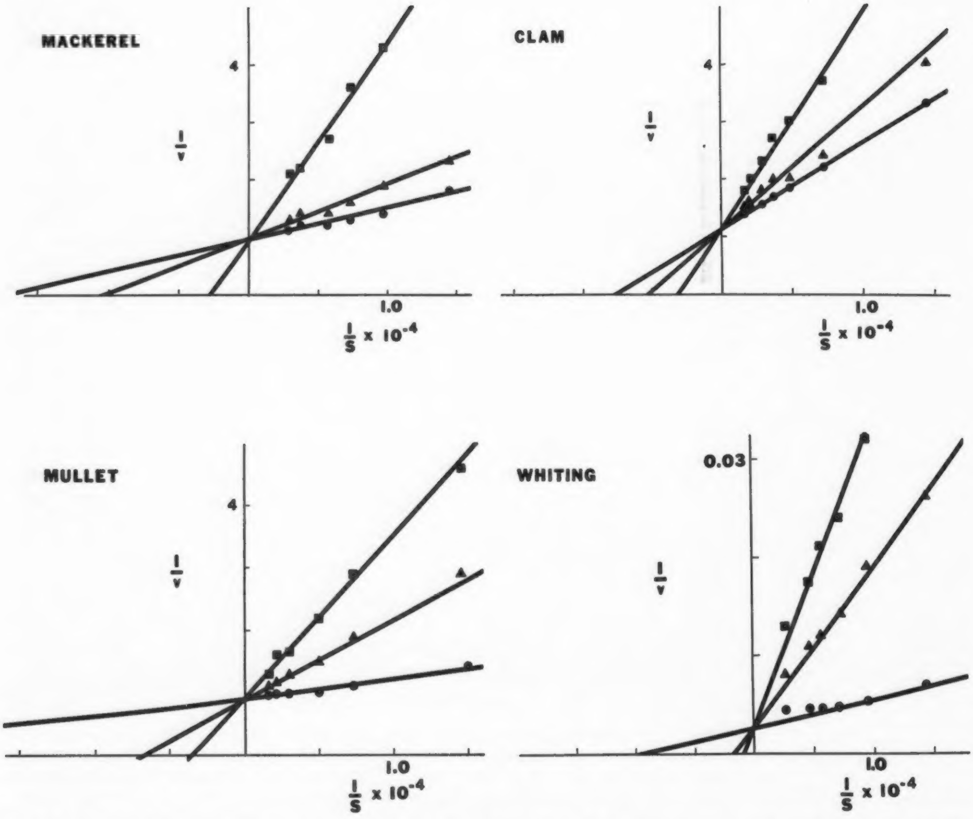

FIG. 3. Effect of puromycin on the activity of arylamidase of mullet, whiting, mackerel, and clam. For mullet and whiting, concentrations of puromycin are ●——● 0.0 μM, ▲——▲ 1.0 μM, and ■——■ 2.5 μM. For mackerel, concentrations are ●——● 0.0 μM, ▲——▲ 0.25 μM, and ■——■ 2.5 μM. For clam, concentrations are ●——● 0.0 μM, ▲——▲ 1.0 μM, and ■——■ 10 μM.

Substrate specificity — The substrate for arylamidase is not known but a determination of the preferred amino acid-β-naphthylamide substrate may indicate the nature of the in situ site of attack. The substrate specificity profiles obtained with nine amino acid-β-naphthylamide substrates and reported as percent relative to the preferred substrate are shown in Fig. 1. The six marine species hydrolyzed neutral and basic naphthylamide substrates but possessed negligible activity against the acidic arylamide tested, aspartyl-β-naphthylamide. In all six marine muscle extracts, the three amino acid-β-naphthylamides hydrolyzed best were lysyl-, alanyl-, and leucyl-β-naphtyhlamides. The mackerel, mullet, and whiting muscle enzymes worked best on alanyl-β-naphthylamide, having specific activities of 55, 74, and 94, respectively. Shrimp and crab enzymes hydrolyzed lysyl-β-naphthylamide at the fastest rate, with specific activities of 23 and 112, respectively. The clam muscle was noted to possess an exceedingly high specific activity against leucyl-β-naphthylamide (763 µg β-naphthylamine released/h per milligram protein). The addition of $-Ch_2-CH_2-NH_2$ to alanyl-β-naphthylamide to increase the chain length (lysyl-β-naphthylamide) greatly affected activity, as did a change in branching from δ (leucyl-β-naphthylamide) to β (isoleucyl-β-naphthylamide).

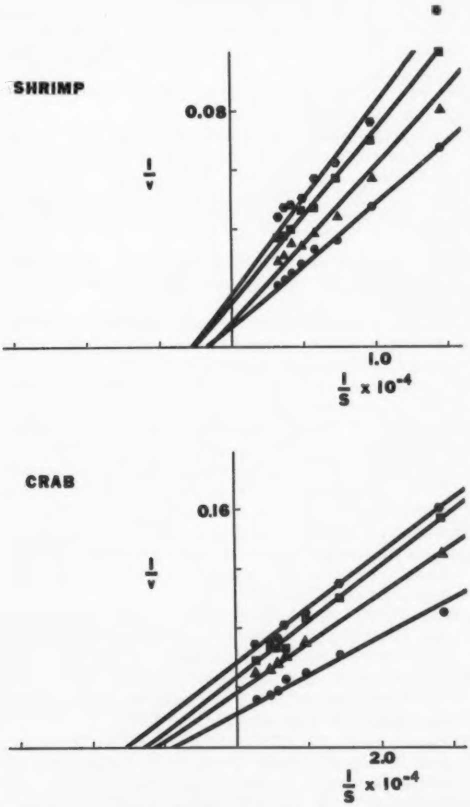

FIG. 4. Effect of puromycin on the activity of arylamidase of shrimp (top) and crab (bottom). For shrimp enzyme, concentrations of puromycin are ●——● 0.0 µM, ▲——▲ 0.001 µM, ■——■ 0.005 µM, and ●——● 0.01 µM. For crab, concentrations are ●——● 0.0 µM, ▲——▲ 0.001 µM, ■——■ 0.01 µM, and ●——● 0.1 µM.

Histochemical staining of acrylamide gels of the mackerel, mullet, whiting, blue crab, and clam extracts indicated a single band of activity (Fig. 2). Positions of the active bands were the same when the gels were incubated in alanyl-, leucyl-, or lysyl-β-naphthylamide, suggesting that the same enzyme was hydrolyzing the different substrates. Gels of the shrimp extract contained two active arylamidase bands, the faster moving band was active against lysyl-, alanyl-, and leucyl-β-naphthylamides; whereas the slower moving band was active only against lysyl-β-naphthylamide. The faster-moving shrimp muscle arylamidase appears to resemble arylamidases reported initially by Ellis (1963) with activity against both neutral and basic arylamide substrates. The slower-moving shrimp enzyme with activity only against lysyl-β-naphthylamide corresponds in this respect to arylamidase B which is active only towards basic substrates (Hopsu et al. 1966). The failure of the fish muscle extracts and muscle extracts from the clam and crab to show more than one active band upon electrophoresis indicates but does not conclusively prove the absence of multiple arylamidases in these muscles.

Multiple arylamidase systems have been observed by gel electrophoresis in *Sarcina lutea* (Behal and

TABLE 1. Kinetic parameters of the six marine muscle enzymes.

Enzyme source	Km_{app} (M)	Ki_{app} (M)	Puromycin concn giving 50% loss of activity (M)
Whiting	5.4×10^{-5}	2.1×10^{-7}	
Mullet	3.1×10^{-5}	3.5×10^{-7}	
Mackerel	3.2×10^{-5}	3.2×10^{-7}	
Clam	1.3×10^{-4}	5.2×10^{-6}	
Crab	1.1×10^{-4}		5.0×10^{-7}
Shrimp	3.4×10^{-4}		1.0×10^{-8}

Carter 1971) and wound tissue (Makinen and Raekallio 1968). The *S. lutea* enzyme had three active components, two of which were electrophoretically similar and had identical *p*H optima and substrate specificities. Makinen and Raekallio (1968) showed that arylamidase of normal tissue was electrophoretically different from wound tissue. Rustow and Hock (1972) showed five active bands in rat liver, two in the pancreas, and four from intestinal mucosa.

Puromycin inhibition — As arylamidases, in contrast to other exopeptidases, are inhibited by puromycin, this property can be used to differentiate the arylamidases from closely related aminopeptidases (Marks et al. 1968). Previous reports have shown the inhibition to be of a competitive nature (Suszkiw and Brecher 1970; Marks et al. 1968). The inhibition curves for the arylamidases from the marine species are shown in Fig. 3 and 4. Using alanyl-β-naphthylamide as substrate, the apparent Michaelis constants (Km_{app}) of the six species, the inhibitor constants of the fish and clam enzymes, and puromycin concentrations resulting in a 50% loss of activity for shrimp and crab enzyme are listed in Table 1.

The enzymes from the three species of fish showed competitive inhibition to puromycin with similar inhibitor constants and similar Km_{app} values. Crab arylamidase showed inhibition approaching uncompetitive behavior. Apparent Michaelis constants for the crab enzyme decreased with an increase in the concentration of inhibitor, showing that in the crude extract, the presence of puromycin may aid in the binding or release of the substrate from the enzyme. The calculated value of Ki_{app} for this enzyme increased with increasing inhibitor concentration, showing a decreasing sensitivity of the enzyme as concentration of puromycin was increased. This might be due to the presence of a protector molecule in the crude extract, causing a need for a higher puromycin concentration than would be expected to inhibit the enzyme to a predefined extent. A Ki value for this enzyme cannot be assigned until the purified enzyme is available.

Arylamidase of shrimp muscle showed the highest Km_{app} value and the greatest sensitivity to the inhibitor. Similarly to the crab enzyme, increasing inhibitor concentrations caused a decrease in the calculated Ki_{app} value. The presence of two active components as seen by gel electrophoresis may influence inhibition behavior.

Since the discovery of arylamidases in 1963, several studies have suggested a proteolytic role for this group of enzymes. Although more work needs to be done on the in vivo function before a definite physiological role is assigned, the presence of arylamidase in the muscle of the marine species examined presents the possibility that these enzymes are potentially influential in muscle autolysis.

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Duration of Toxicity Tests for Establishing "No Effect" Concentrations for Copper with Brook Trout (*Salvelinus fontinalis*)

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MCKIM, J. M., AND D. A. BENOIT. 1974. Duration of toxicity tests for establishing "no effect" concentrations for copper with brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 31: 449–452.

Exposure of brook trout (*Salvelinus fontinalis*) to sublethal concentrations of copper from yearlings through spawning to 3-mo juveniles was found sufficient to establish a "no effect" concentration. No adverse effects were found on survival, growth, or reproductive capacity of trout exposed in a second generation from egg through spawning to copper concentrations (about 9.4, 6.1, and 4.5 μg/liter) which had caused no adverse effects on their parents when exposed to similar concentrations from yearlings to spawning. Nor were any adverse effects found on survival or growth of their offspring when exposed to the same concentrations from hatching to age 90 days. Tissue residue analyses indicated no copper accumulation in gill, liver, kidney, or muscle of either first- or second-generation trout exposed to the various copper concentrations, and residues in unfertilized eggs from exposed females were similar to those in females not exposed to added copper.

MCKIM, J. M., AND D. A. BENOIT. 1974. Duration of toxicity tests for establishing "no effect" concentrations for copper with brook trout (*Salvelinus fontinalis*). J. Fish. Res. Board Can. 31: 449–452.

On a découvert que l'exposition d'ombles de fontaine (*Salvelinus fontinalis*) à des teneurs sublétales de cuivre entre le stade d'alevin de moins d'un an, passant par la fraie, jusqu'au stade juvénile de 3 mois est suffisant pour déterminer la teneur à "effet nul." Nous n'avons pas observé d'effets défavorables sur la survie, la croissance ou le pouvoir reproducteur d'ombles exposés à la seconde génération, de l'oeuf jusqu'à la fraie inclusivement, à des teneurs de cuivre (environ 9.4, 6.1 et 4.5 μg/litre) qui n'avaient pas eu d'effets défavorables sur les parents, après que ceux-ci aient été exposés à des teneurs semblables entre le stade d'alevin de moins d'un an et la fraie. Nous n'avons pas trouvé non plus d'effets défavorables sur la survie ou la croissance de leurs descendants lorsque ceux-ci ont été exposés aux mêmes teneurs entre l'éclosion et l'âge de 90 jours. Les analyses de résidus tissulaires ne révèlent pas d'accumulation de cuivre dans la branchie, le foie, le rein ou le muscle, ni chez les ombles de première ni de seconde génération exposés aux diverses teneurs en cuivre. Les résidus dans les oeufs non fécondés de femelles exposées sont semblables à ceux de femelles non exposées.

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THIS study was designed to evaluate the validity of exposing brook trout (*Salvelinus fontinalis*) from yearlings through spawning to early juveniles for establishing "no effect" copper concentrations. McKim and Benoit (1971) exposed brook trout to copper from yearlings to spawning, the offspring until they were yearlings, and newly hatched offspring from unexposed parents until they too were yearlings. The results indicated toxic effects on both groups of offspring at 32.5 and 17.4 μg/liter copper, but not at 9.5 μg/liter or below. There was, however, no evidence to discount the possibility that adverse effects of the lower copper concentrations might appear in these fish after exposure for a cycle from eggs to spawning or in their offspring. To check this possibility, the exposure of yearlings from the previous study from parents exposed to added copper and those not exposed was continued. This allowed testing from eggs to spawning for effects of sublethal concentrations of copper (about 9.4, 6.1, 4.5, and 2.7 [control] μg/liter) on fish whose parents had not been exposed previously to added copper (group I) and on fish whose parents had been exposed for 8 mo to similar concentrations (group II). An evaluation was made for both groups of the effects of copper on survival and growth from yearlings to spawning, on numbers of offspring, and on survival and growth of the offspring from spawning to 90 days after hatching. Also, accumulation of copper in tissues and eggs of both groups of fish was investigated. The study extended from January 1, 1970, to April 15, 1971.

Methods and materials — The proportional diluter, exposure system, and methods of egg incubation, determining hatchability, measuring chemical and physical water characteristics, and determining copper concentrations in the test water, except as described here, were described by McKim and Benoit (1971).

Water quality characteristics (mg/liter except as indicated) were:

Characteristic	No. of observ.	Mean	Range
Temp. (C)	434	9.6±0.23*	5–18
Dissolved O_2	282	9.1±0.24	3.6–12.3
*p*H	64	—	6.9–8.8
Hardness (as $CaCO_3$)	59	44.0±1.38	40–46
Alkalinity (as $CaCO_3$)	61	42.3±0.28	39.5–48.0
Acidity	61	3.9±0.38	2.2–7.9

*95% confidence limit.

Two stainless steel tanks (30.5 × 39.6 × 182.9 cm, water depth 30.5 cm) were maintained at each of the three test copper concentrations (one for each group of fish), and one for the controls since the control fish for the two groups were from the same parental stock with regard to copper exposure. The measured copper concentrations (Table 1) were based on 64 measurements made throughout the experiment. Initially, 20 fish were used per tank. Two weeks prior to spawning (October 25, 1970) each tank was divided crosswise with a screen into compartments A and B and, except for shortages of females in some tanks, two ripening males and four ripening females were randomly established in each compartment. The remaining fish were discarded. Growth studies on the yearlings were also terminated 2 wk prior to spawning. Prior to spawning, water temperatures ranged from 9–15 C.

During spawning, egg incubation, and the 90-day growth studies, water temperatures were maintained at 9 C ± 1°. The incubation apparatus consisted of oscillating .12-liter (4-ounce) glass jars with nitex screen bottoms designed after those of Mount (1968). Fifty fertilized eggs from each of the first five spawnings in each compartment were selected at random for hatchability measurements. If more than five spawnings occurred, the fertilized eggs from the later spawnings were incubated for 12 days only and discarded after a check for viability. After hatching ceased (January 4–February 4, 1971) all alevins in each jar were weighed, measured, and randomly reduced to 25 per jar, where they remained for 3 wk. Two hatching jars were then randomly selected from each compartment and the alevins from these jars released into separate growth chambers for the growth studies. Growth chambers were 38.1 cm long, 12.7 cm wide, and 17.8 cm deep (water depth 12.7). Juvenile weight was determined on all fish 90 days after hatching.

All fish were fed frozen trout pellets (Oregon Moist[1]) that had been checked for chlorinated hydrocarbons. Fish up to 3 mo old were fed five times daily and older fish twice daily.

The fish were treated twice with neomycin and twice with chloromycetin to eliminate a bacterial disease caused by *Aeromonas liquefaciens*. Four mortalities (three control fish, one male and two females, and one female fish at 6.2 μg/liter), which occurred during spawning, were attributed to this pathogen; some mortality at spawning is not uncommon among salmonids.

Gill, liver, kidney, gonad, and muscle samples were dissected from the adult fish after spawning and frozen for copper-residue analyses (Leonard 1971). The numbers of males and females that had matured in each compartment were determined, by examination of the gonads, after spawning ceased. Also, egg samples of 40 eggs each were taken from the body cavities of several mature females of each group from all test copper concentrations and the control for determination of copper residues. Copper residues were also measured on 25 fertilized control eggs incubated 3 wk in the highest copper concentration and in the control.

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[1] Moore and Clark Co., LaConner, Wash. 98257, USA. Mention of trade names does not constitute endorsement.

Spawning data and adult and juvenile weights were subjected to an analysis of variance with an F test of significance ($P = 0.05$).

Results — No effects on survival, growth, or number of progeny were seen in either group of brook trout exposed to the various concentrations of copper ($P = 0.05$) (Table 1). Nor were adverse effects seen on egg hatchability or on growth or survival of offspring of either group except for decreases ($P = 0.05$) in egg hatchability and juvenile weight at 9.4 and in juvenile weight at 4.5 µg/liter (Table 2). However, when we considered eggs per female, hatchability, alevin–juvenile survival, and juvenile weight as a group, the estimated total weights of 90-day-old young per female for the two groups at all copper concentrations were similar (Table 2).

The ratio of estimated total weight of 90-day-old young per female between fish exposed to added copper and controls (Table 2) allowed comparison of data in the previous study (McKim and Benoit 1971) with the present data. At the copper concentrations of 9.4 µg/liter and below in both studies,

TABLE 1. Survival and growth from age 12 mo to spawning, and egg production, of brook trout (*Salvelinus fontinalis*) exposed continuously to sublethal concentrations of copper: group I, fish from parents not previously exposed, and group II, fish from parents exposed (McKim and Benoit 1971) to similar concentrations from age 12 mo to spawning.

	Copper (µg/liter)													
	9.0±0.4[a]		9.7±0.8		6.1±0.3		6.2±0.4		4.4±0.1		4.7±0.3		2.7±0.1 (control)	
	I		II		I		II		I		II		I	
Item	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B	A	B
Survival to spawning (%) Jan. 1, 1970–Sept. 25, 1970	95(20)[c]		94(16)		100(20)		95(18)		95(20)		91(11)		100(20)	
Initial size (Jan. 1, 1970)														
Length (*cm*)	16±0.9[a](20)		15±1.6(16)		14±1.4(20)		14±0.9(18)		15±1.4(20)		15±1.3(11)		14±0.9(20)	
Weight (g)	42±7.9(20)		34±7.9(16)		30±9.4(20)		31±6.4(18)		35±8.4(20)		37±11.3(11)		33±6.5(20)	
Size at spawning (Sept. 25, 1970)														
Length (*cm*)	24±1.4(19)[d]		25±1.7(15)		25±3.7(20)		26±1.5(17)		24±0.9(19)		24±3.0(9)		25±3.3(20)	
Weight (g)	172±28.8(19)		200±48.4(15)		180±32.7(20)		195±30.2(17)		164±21.1(19)		192±62.5(9)		173±25.7(20)	
No. mature males	2	4	2	2	2	2	3	2	3	2	2	2	2	1
No. mature females	3	2	3	4	4	4	3	3	3	3	2	3	4	2
No. females spawning	2	2	3	3	2	4	3	2	3	3	2	3	3	2
Mean spawnings per female	1.0	1.5	2.0	3.0	2.5	1.5	1.3	2.0	2.0	3.3	4.0	3.7	2.3	1.5
Mean viable eggs spawned per female	389	283	406	469	392	280	296	169	319	507	802	828	507	233

[a] Mean and 95% confidence limits.
[b] Duplicate spawning compartments.
[c] Number of fish initially exposed.
[d] Number of fish remaining.

TABLE 2. Estimated total weights of 90-day-old brook trout produced per female at various copper concentrations for the two groups (Table 1) of fish.

Copper concn µg/liter	Group of fish	Estimated[a] eggs/female	Hatchability (%)	Alevin–juvenile survival to 90 days (%)[c]	Individual juvenile weight[d] at 90 days (g)	Estimated total weight of 90-day-old young/female (g)	Ratio of estimated total weight of 90-day-old young/female (Exposed: Control)	
							This study	McKim and Benoit(1971)
2.7	control	335	92(10)[b]	70	0.57±0.04(67)	122.97		
4.5	I	370	96(10)	68	0.59±0.07(66)	142.51	1.16	1.20
	II	700	90(10)	64	0.42±0.05[e](59)	169.34	1.38	–
6.1	I	315	91(10)	77	0.53±0.05(75)	116.98	.95	1.07
	II	215	82(8)	88	0.61±0.07(85)	94.63	.77	–
9.4	I	300	99(5)	67	0.53±0.06(67)	105.47	.86	.85
	II	452	65[e](10)	83	0.38±0.05[e](77)	92.67	.75	–
17.4		–	–	–	–	–	–	.59
32.5		–	–	–	–	–	–	.07

[a] Number of viable eggs produced per spawning female × the probability of spawning (i.e. total spawners/total females = .8605).
[b] Number of spawnings checked for hatchability.
[c] 100 fish exposed in each test.
[d] Mean and 95% confidence interval.
[e] Difference from control value significant at $P = 0.05$.

TABLE 3. Copper residues[a] (μg/g) in eggs and tissues of brook trout exposed to copper for 24 mo (values for groups I and II combined).

	Water copper (μg/liter)			
Tissue	9.4	6.1	4.5	2.7 (Control)
Gill	6.7±2.16(12)[b]	4.0±1.2(12)	4.0±1.0(12)	5.0±3.3(6)
Liver	238.4±89.5(12)	207.8±67.3(11)	237.6±146.4(12)	239±109.1(6)
Kidney	16.5±7.4(12)	12.6±3.0(11)	15.2±6.1(12)	19.7±14.1(5)
Muscle	17.0±6.6(10)	8.2±2.9(11)	6.9±2.7(11)	15.3±6.3(4)
Eggs (from body cavity of ripe fish)	6.8±2.1(5)	8.4±2.1(7)	5.7±0.4(3)	9.4±3.6(4)

[a] Means and 95% confidence limits.
[b] Number of samples analyzed.

variation from unity in the ratios was not great. There was, however, a large decrease (41–93%) in the ratios for the fish in the previous study at concentrations of 17.4 μg/liter and above (Table 2). This reduction was indicative of a later loss of all fish at 17 μg/liter copper and above, as described by McKim and Benoit (1971).

At the concentrations tested, copper did not accumulate in the various tissues of the fish (Table 3). Also, the copper concentrations in tissues did not differ between males and females.

Egg samples taken from the body cavities of trout exposed to the various concentrations of added copper and the control all contained essentially the same amounts of copper (Table 3). When fertilized control eggs were incubated for 3 wk in water containing 9.4 μg/liter copper, copper residues increased to 27.8 ± 0.7 from 6.3 ± 1.7 μg/g.

Discussion — The results of this study showed that 9.4 μg/liter copper and less had no adverse effects on either first or second generation brook trout exposed in the laboratory through a cycle from eggs to spawning. This confirmed the previous findings of McKim and Benoit (1971) on exposure of brook trout to similar and higher copper concentrations, which indicated no adverse effect of exposure from yearlings through spawning to 3-mo juveniles at 9.4 μg. This strengthens the assumption that long-term exposure of appropriate stages in the life history may be sufficient to demonstrate the "no effect" concentrations of other metals.

The lack of accumulation of copper in fish tissues in water with low copper concentrations (Table 3) was noted earlier in the brown bullhead (*Ictalurus nebulosus*) by Brungs et al. (1973). They found that after 2 yr exposure the bullhead accumulated copper only at water concentrations above 16 μg/liter. Above this copper level they postulated that the copper excretory mechanism was probably overloaded and copper accumulated within the body, which contributed to later toxic effects. This also appeared plausible for the brook trout, since we found no accumulation of tissue copper and no toxic symptoms over the 2-yr period at 9.4–4.5 μg/liter copper. The copper residues in unfertilized eggs indicated that no copper was passed from the parent to the egg at 9.4 μg/liter and below. However, copper concentrations in fertilized eggs increased slightly after a 3-wk incubation period in water containing 9.4 μg/liter. This copper may be bound to the outer shell membrane and not contribute to an incorporation of copper within the embryo. If there was an increased accumulation in the embryo it had no direct toxic effect, and the initial accumulation was lost with time.

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Portuguese and Japanese Oysters are the Same Species

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MENZEL, R. W. 1974. Portuguese and Japanese oysters are the same species. J. Fish. Res. Board Can. 31: 453–456.

Indistinguishable prodissoconch and adult shells, ease of hybridization, and normal meiosis and mitosis in the hybrids support the contention that the Portuguese oyster *Crassostrea angulata* and the Japanese oyster *C. gigas* are the same species. By rules of priority the Portuguese is a subspecies of the Japanese oyster. Two hypotheses are given to explain the widely disjunct distribution, by both species arising from a common ancestor or by undocumented introduction. Both oysters are of considerable commercial importance and crossing the two should result in progeny that can be selected for superior commercial traits.

MENZEL, R. W. 1974. Portuguese and Japanese oysters are the same species. J. Fish. Res. Board Can. 31: 453–456.

Une prodissoconque et des valves adultes indiscernables, facilité d'hybridation, et méiose et mitose normales chez les hybrides appuient la prétention que l'huître portugaise, *Crassostrea angulata*, et l'huître japonaise, *C. gigas*, sont une même espèce. Selon les règles de la priorité, l'huître portugaise est une sous-espèce de l'huître japonaise. L'auteur met de l'avant deux hypothèses pour expliquer leur répartition largement discontinue: ancêtre commun pour les deux espèces ou introduction non documentée. Les deux huîtres ont une importance commerciale considérable, et, en croisant les deux, on devrait obtenir une progéniture pouvant être sélectionnée pour ses qualités commerciales supérieures.

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THE Portuguese oyster *Crassostrea angulata* (Lamarck) and the Japanese oyster *C. gigas* (Thunberg) are both important commercial species. The center of the population for each species is about 40° N, lat. but they are from 130 to 140° long. apart, almost on opposite sides of the world. Ranson (1948, 1960, 1967) has long maintained that the two were the same species, because his studies showed that the late prodissoconch shells of the two were indistinguishable, whereas he could separate other species on differences in the larval shells. Korringa (1952) questioned Ranson's claim, thinking it unlikely that *C. gigas* could have been introduced to Portugal before *C. angulata* was known to be growing there. I do not know what the earliest record for *C. angulata* in Portugal may be, but Dean (1893) says that this species has been growing near Lisbon "since time immemorial." Korringa (1952) suggested that laboratory crossing should determine the validity of Ranson's assertion. Later Imai and Sakai (1961) and Menzel (1968, 1971, 1972) reported that the two species crossed readily and the hybrids were easily reared.

This report presents additional data on the affinities of the two species, offers two possible explanations for the disjunct distribution, and supports the claim of Ranson that both are the same species.

Mature *C. angulata* were obtained from England, originally from France where the Portuguese oyster became established about a century ago, and *C. gigas* from the State of Washington, cultivated from seed oysters imported from Japan. The rearing of the larvae followed the techniques of Loosanoff and Davis (1963), as reported previously (Menzel 1968). Fertilization was up to 95% in the 12 reciprocal crosses made, which is as high a percentage as when each species is selfed by the stripping technique (mixing of gametes from sacrificed parents) used. Six attempts were made to rear the larvae and two crosses of ♀ *C. angulata* × ♂ *C. gigas* and one of the reciprocal ♀ *C. gigas* × *C. angulata* were reared through metamorphosis and attachment. In two of the other three attempted rearings a few of the larvae lived for 10 or more days.

The hybrid oyster spat were reared in tanks in the laboratory, with flowing sea water pumped continuously from the adjacent bay. The effluent from the tanks was prevented from returning to the bay waters to preclude accidental introduction of the species and of pests. The exotic species used for brood oysters were kept in similar tanks with

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controlled effluents. Length and width were measured monthly with vernier calipers to the nearest 0.5 mm (Table 1). Thickness (between the valves), volume, and weight could not be measured because the young oysters were attached to cultch material. The largest hybrid spat of each reciprocal measured about 75-mm length and 45-mm width in October 1968. A move to a new laboratory site in November 1968 resulted in total mortality before the following spring, probably because of unsuitable salt water supply.

On two occasions during the second fall after attachment, the spat (now 1 yr old) having become sexually mature, spawned spontaneously when returned to the water after examination. No attempt was made to rear any of the resulting F_2 embryos, but eggs and early cleavage stages (two to four cells) were preserved for cytological examinations of meiosis and mitosis, using the squash technique with examination under phase contrast optics (Menzel and Menzel 1965). Photomicrographs were taken with a Polaroid camera attachment, and drawings made with the aid of a camera lucida. Meiosis in the F_1 hybrids and mitosis in the F_2's appeared normal (Fig. 1). Normal mitosis in the F_1 hybrids was reported previously (Menzel 1968).[1]

Oysters, especially in the genus *Crassostrea*, have been termed ecomorphic (Thomson 1954) in that shell shape is strongly influenced by environmental conditions. Shell morphology, as well as pigmentation, was very similar, however, in the *C. angulata* obtained from England and *C. gigas* obtained from Washington. *Crassostrea gigas* have developed rather distinct races in northern and southern Japan, so different in maximum size and pigmentation that they were called different specific names (Kahn 1950) until Imai et al. (1950) crossbred the two, which led to the general acceptance of the two types as varieties of the same species. I do not know whether races of the Portuguese oysters have developed or not. Certainly the *C. angulata* and *C. gigas* that I secured are more alike morphologically than the two races of the Japanese *C. gigas* are to each other.

The identical prodissoconch shells and the similarity of the adult shells, the ease of hybridization and the normal chromosomal behavior in the hybrids, all suggest that the Portuguese and Japanese oysters are the same species. Only their occurrence on opposite sides of the world supports the designation as two species.

The widely disjunct distribution of virtually identical populations may be explained in several ways. Stenzel (1971) said that *C. angulata* and *C. gigas*, as well as *C. cattuckensis* (Newton and Smith) (= *C. madrasensis* Preston) found on Indian shores, all derived from the fossil *C. gryphoides* (von Schlotheim), which occurred in the Miocene from Portugal–Spain around to Asia Minor and into Japan. At this time a continuous warm shallow sea (Tethys) existed over the wide area, allowing for the wide range of the species. Toward the end of the Miocene and later, tectonic events produced land barriers, isolating three populations, resulting in three species.

Stenzel's (1971) hypothesis requires that the two species have remained morphologically identical and genetically compatible during 10 million yr or more of isolation. H. B. Stenzel (personal communication) reasserts that both *C. angulata* and *C. gigas* descended from *C. gryphoides* of the Miocene seas by purely mechanical, geographical, geological separation and that the species have not drifted apart genetically to an appreciable amount. The Japanese *C. gigas*, however, has developed into two morphologically distinct races and such genetic stability of disjunct populations and lability of adjacent populations may seem contradictory.

It seems highly unlikely that *C. gigas* could have been transported to Portugal before *C. angulata* is known to occur, as Korringa (1952) remarks. One alternate hypothesis is that *C. angulata* was introduced to Japan. The 16th and 17th centuries were periods of great explorations by the Portuguese. The first record of a visit by Caucasians to Japan was by Portuguese sailors in 1542 (Prestage 1933). This or subsequent voyages could have introduced *C. angulata* by accident (attached to ship bottoms?) or by design. Oyster culture started in Japan in the early 1600s. Kahn (1950) cites three legends for the start of Japanese oyster culture, all dating from the early 1600s. No evidence has been found in the literature that *C. gigas* occurred in Japan before this.

If the Portuguese oyster had been introduced into Japan in the late 1500s or early 1600s, there would have been ample time and opportunity for the species to become established and also to develop distinct races, especially as man was aiding the oysters by supplying cultch material for larval attachment upon metamorphosis and probably was increasing the range through transplanting. A single mating will produce millions of offspring. Although Hirase (1932) lists 20 species of oysters in Japanese waters (some now considered synonymous), only *C. gigas* occurs in shallow estuarine

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[1] *Crassostrea angulata* and *C. gigas* were hybridized with *C. virginica*, the native Eastern North American species. Meiosis in the F_1 hybrids and mitosis in the F_2 hybrids of these two crosses showed gross anomalies, indicating that both *C. angulata* and *C. gigas* are genetically separate from *C. virginica*.

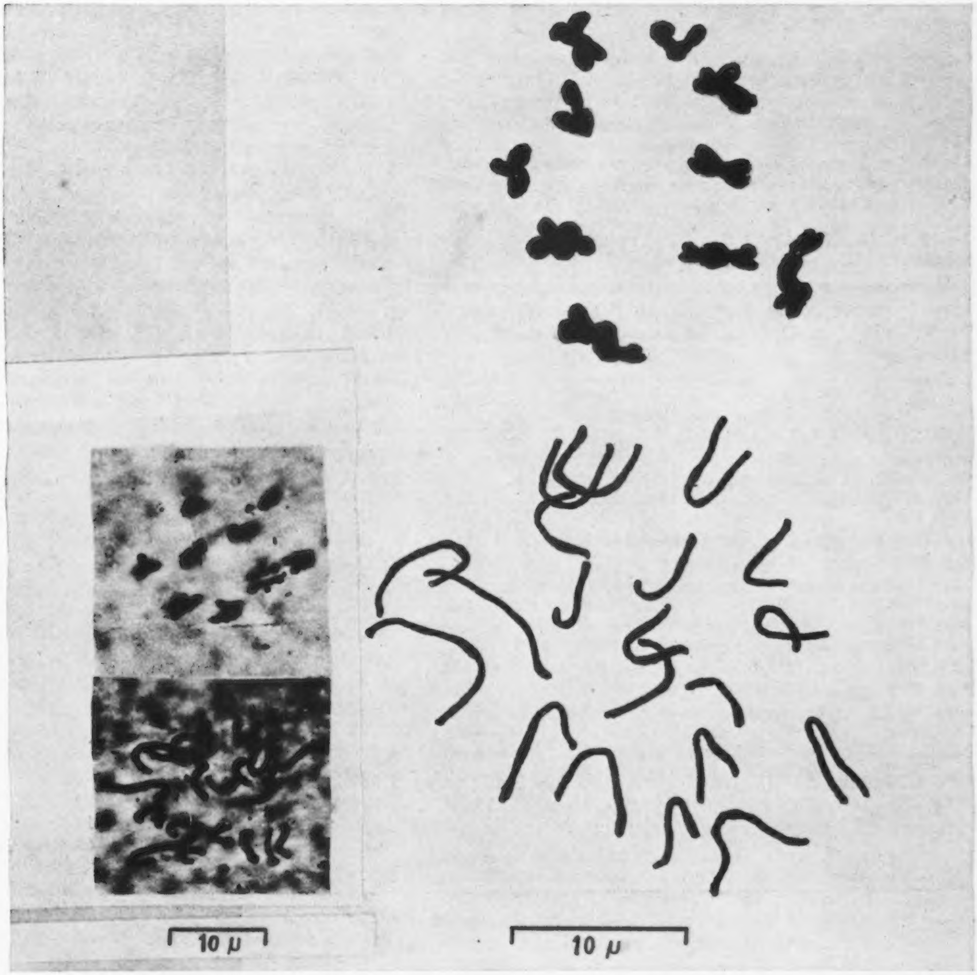

FIG. 1. Photomicrographs and camera lucida drawings of meiosis (N=10) in F_1 hybrid and mitosis (2N =20) in F_2 hybrid of *Crassostrea angulata* × *C. gigas*.

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Table 1. Number, mean size (mm), and percentage of mortality of laboratory-grown hybrids, *Crassostrea angulata* × *C. gigas*. Period September 1967–October 1968.

	♀ *C. angulata* × ♂ *C. gigas*[a]			♀ *C. gigas* × ♂ *C. angulata*[b]		
		Size	% mortality cumulat.		Size	% mortality cumulat.
Date exam.	#	L[c] × W[c]		#	L[c] × W[c]	
Sept. 1967	109	4.5×3.0	–	33	4.5×4.0	–
Oct. 1967	95	11.0×9.5	13	28	12.0×9.0	15
Nov. 1967	87	14.0×12.0	20	28	12.5×11.0	15
Dec. 1967	87	14.5×12.5	20	27	12.0×11.5	18
Jan. 1968	87	14.5×12.5	20	26	12.5×11.5	21
Feb. 1968	84	14.5×12.0	23	26	12.5×11.5	21
Mar. 1968	83	14.5×12.0	24	26	12.5×11.5	21
Apr. 1968	81	21.0×17.0	26	26	17.0×13.5	21
May 1968	79	32.0×27.5	28	26	21.0×18.0	21
June 1968	78	40.5×31.5	28	24	37.5×28.5	27
July 1968	73	44.0×31.5	33	24	46.5×34.5	27
Aug. 1968	70	46.0×35.0	36	22	46.5×34.0	33
Sept. 1968	68	46.5×34.5	38	22	46.5×44.5	33
Oct. 1968	65	51.5×37.5	40	22	51.0×37.0	33

[a] ♀ *C. angulata* × ♂ *C. gigas* — attached on July 28 and August 5, 1967;
[b] ♀ *C. gigas* × ♂ *C. angulata* — attached August 18, 1967.
[c] Length (L) is longest axis from umbo to posterior; width (W) at approximate right angles to length.

areas and has no competition from other species of oysters.

The introduction of species to new areas, especially where there is an unoccupied niche, with subsequent population explosions, has been documented many times. The Portuguese and Japanese oysters seem especially adaptable to colonizing new areas. The Portuguese oyster was introduced into the Gironde Estuary in France in the mid-1800s, and spread rapidly northward until stopped by water temperatures too low to allow for spawning (Yonge 1969). The Japanese oyster has been transplanted widely for commercial cultivation. This species became established within a few years after introduction to Australia (Thomson 1952, 1959). Recently *C. gigas* was found in New Zealand with no explanation except "by accidental passive dispersal" (Anon. 1972). Small seed oysters are regularly transplanted to the northwest coast of North America from Japan, supporting a large commercial fishery. In most of the area the water temperature remains too low to allow for successful spawning. There is, however, a thriving reproducing population in an area of British Columbia, where hydrographic conditions are suitable. The Canadian oyster fishermen are using this area as a source of seed oysters, with curtailment of seed from Japan (Bourne 1972; Quayle 1969).

Neither of the hypotheses is entirely satisfactory but regardless of the explanation for the distribution of the two oysters, the evidence supports the conclusion that *C. angulata* and *C. gigas* are the same species. If the hypothesis is correct, that both *C. angulata* and *C. gigas* derived from the fossil *C. gryphoides*, then the two have remained genetically compatible and morphologically similar for up to 10 million yr, and thus the fossil *C. gryphoides* is also the same species. It remains for future investigation to determine if the Indian *C. cattuckensis* is the same as *C. angulata* and *C. gigas*.

According to the rules of priority all the oysters should be designated *Crassostrea gigas* (Thunberg 1793), which has priority over *C. angulata* (Lamarck 1819), *C. gryphoides* (von Schlotheim 1813), assuming that both arose from it, and *C. cattuckensis* (Newton and Smith 1912), if this latter species proves to be in the same complex. To be conservative, the Portuguese and Japanese oysters should be designated as subspecies — *C. gigas angulata* for the Portuguese and *C. gigas gigas* for the Japanese oyster. Undoubtedly the oysters from Japan and Portugal have geographically separate gene pools, and in addition, this terminology would be of convenience for workers with oysters.

Both the Portuguese and Japanese oysters are of considerable importance. They have been separated for a least 300 and possibly for 10 million yr and recombination of the gene pools by hybridization might result in heterosis. Selection for traits of commercial importance should result in superior oysters for commercial exploitation.

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Color and Some Composition Changes in Ocean Perch (*Sebastes marinus*) Held in Refrigerated Sea Water With and Without Carbon Dioxide

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LONGARD, A. A., AND L. W. REGIER. 1974. Color and some composition changes in ocean perch (*Sebastes marinus*) held in refrigerated sea water with and without carbon dioxide. J. Fish. Res. Board Can. 31: 456–460.

Skin color of ocean perch (*Sebastes marinus*), measured instrumentally, was at least as good when the fish were held up to 12 days in refrigerated sea water (RSW) at –1 C as when they were held in ice. Adding carbon dioxide to the RSW generally enhanced skin color retention. The loss in total extractable carotenoid pigments was not much affected by the storage conditions. The fish stored in RSW with CO_2 were kept in better condition, as measured by trimethylamine production in the fillet tissue, than in ice or RSW. The ionic diffusion, sodium uptake, and potassium loss between the fish and the RSW did not appear excessive but did not reach equilibrium in 12 days storage.

LONGARD, A. A., AND L. W. REGIER. 1974. Color and some composition changes in ocean perch (*Sebastes marinus*) held in refrigerated sea water with and without carbon dioxide. J. Fish. Res. Board Can. 31: 456–460.

La couleur de la peau du sébaste atlantique (*Sebastes marinus*), mesurée au moyen d'un instrument, est au moins aussi bonne quand le poisson a été maintenu jusqu'à 12 jours dans l'eau de mer réfrigérée (EMR) à –1 C que quand il a été conservé dans la glace. L'addition d'acide carbonique à l'EMR favorise généralement la rétention de la couleur de la peau. Les conditions d'entreposage n'affectent pas beaucoup la perte des pigments caroténoïdes extractibles totaux. Les poissons entreposés dans l'EMR additionnée de CO_2 se conservent en meilleure condition, telle que mesurée par la production de triméthylamine dans le tissu des filets, que ceux conservés dans la glace ou dans l'EMR. La diffusion ionique, l'absorption de sodium et la perte de potassium entre le poisson et l'EMR ne semblent pas excessives, mais n'ont pas encore atteint un état d'équilibre après entreposage de 12 jours.

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IN shipboard trials on use of refrigerated sea water (RSW) for transport of Atlantic cod (*Gadus morhua*), ocean perch (*Sebastes marinus*), and winter flounder (*Pseudopleuronectes americanus*) (Longard and Regier 1971) the skin of the ocean perch tended to become bleached. Because ocean perch fillets are sold with the red skins on, color is an important factor in quality assessment. RSW with added CO_2 was used with some success to preserve the eating quality of yellowtail rockfish (*Sebastes flavidus*) (Barnett et al. 1971). This combination was studied concerning potential for use with ocean perch to preserve skin color as well as flesh quality. It was expected that the added CO_2 would help to decrease the contact with oxygen and thus prevent oxidative bleaching of the skin pigments.

Methods — For these experiments, a small RSW system (Fig. 1) was assembled for use in the laboratory. Two 150-liter (35-gal) plastic tubs were insulated for use as the storage tanks on two parallel systems. Sea water was pumped from the top of each tank through hose to coil type polyethylene tubing heat exchangers. From the heat exchanger the water was returned to the bottom of the tanks through a perforated diffusion ring at a flow rate of about 16 liters/min (4 gal/min). The heat exchanger coils were immersed in a tank of refrigerated glycol–water mixture kept at about -4 C by a thermostatically controlled freon hot gas bypass system on a commercial refrigeration compressor. This arrangement maintained the recirculating sea water at about -1 C.

A hypodermic needle was inserted in a rubber connection in one of the water lines leading to the heat exchanger after the pump. CO_2 was bled into the flowing RSW at about 1 liter/min.

Four experimental runs were made in which fish were caught by a research vessel and brought to the laboratory in sea water within 6 h. Some of the fish were still alive on landing. About 34 kg (75 lb) of the round fish were placed in ice in an insulated plastic container, in a manner similar to normal shipboard icing of ocean perch. Fifty-six kg (125 lb) of the round fish were placed in each of the storage tanks with enough filtered sea water to give a ratio of one part fish to one part water. Throughout the experimental period, the ratio was maintained by removing water as well as fish at each sampling time. To keep the fish immersed, a perforated plastic cover was inserted below the intake pipe in each tank.

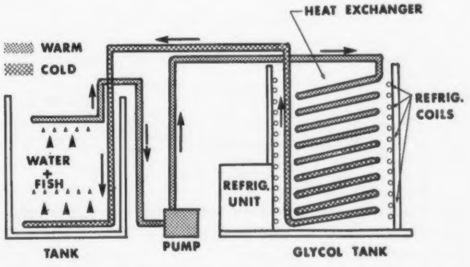

FIG. 1. Experimental recirculating refrigerated seawater system.

Six fish were removed from each of the storage conditions for analyses every few days to the maximum of 12. The appearance of the fish including the gills, eyes, vent, and slime were noted and the fish were then filleted. The 12 fillets in each sample were sealed in transparent nylon–saran–polyethylene-laminated bags and the skin color was measured on a Gardner Color Difference Meter. Readings were made at three locations on each fillet: head end, middle, and tail end along the lateral line. The fillets were then skinned and the meat was ground, mixed, frozen, and held at -26 C until chemical analyses could be done.

The skin was cut into 1-cm wide strips for total carotenoid determination. The skin samples were extracted by the method of Saito and Regier (1971) and the absorbance of the final petroleum ether solution at 460 nm was used as the measure of the carotenoids. The results were calculated as μg lutien/cm^2 skin to minimize the effect of skinning method variations.

The trimethylamine in the samples was determined by the method of Dyer (1959) using 100-g samples of ground fish. For sodium and potassium analyses, 5-g samples were dried in crucibles in a vacuum oven at 70 C and ashed in a muffle furnace at 550 C. The ash was dissolved in concentrated HCl, filtered, and diluted and the sodium and potassium determined by flame emission spectrophotometry.

The Gardner meter gives results in the Y, X, and Z tristimulus values in the CIE (Commission International de l'Eclairage) color space units. These values were converted into the L, a_L and b_L coordinates which relate more closely to visual responses (Hunter 1942). The L scale, or dimension, indicates the range in amount of reflected light. The a_L dimension indicates redness with positive values and greenness with negative values, whereas the b_L dimension indicates yellowness with positive values and blueness with negative values. All the samples had positive a_L and b_L values.

Results and discussion — Upon visual inspection after several days in RSW the fish showed changes that would have been taken to indicate advanced spoilage if they had been stored in ice. The eyes were cloudy and the gills were bleached and slightly slimy. The skin color, however, appeared to be brighter even after 8–10 days. The iced fish tended to have a blotchy appearance being more bleached where they had been in contact with each other or with the ice. The inspection criteria for the quality of ocean perch will thus need revision for judging RSW-stored fish.

The CO_2-treated water was much lighter in color than the untreated water and did not have the putrid odor which the untreated water exhibited after 1 wk. Throughout the experiments the *p*H of the CO_2-treated water was 5.5 and that of the untreated RSW about 6.7.

The TMA nitrogen values for the fish in RSW rose somewhat earlier than in the iced fish but the RSW + CO_2-stored fish exhibited lower values (upper two curves, Fig. 2). In an experimental run in which the refrigeration system broke down at 4 days (bottom curve, Fig. 2) and the RSW systems warmed up, the addition of CO_2 was effective in delaying the TMA production as long as the continuous iced storage. The inhibition of the TMA production seemed to be an effect of the *p*H. Nadeau (1940) reported that, in fish muscle juice maintained at 0 C, the production of volatile nitrogen (or TMA) and bacterial activity diminished gradually as the acidity was increased until *p*H 5 was reached. King and Nagel (1967) also demonstrated the inhibitory effects of CO_2 on bacterial growth and Castell and Snow (1949) showed that the enzymic reduction of trimethylamine oxide became insignificant below *p*H 6.0.

The RSW-stored ocean perch generally kept their skin color better than the iced fish (Fig. 3). In the four experiments the a_L or redness values were generally higher for the RSW than for the iced fish. Of the two treatments with RSW, that with the CO_2 tended to preserve higher a_L values. The L (reflectivity) and b_L (yellowness) did not appear to change appreciably from the original values of about 50 and 12, respectively. There generally was some loss in the total carotenoids on storage, but there was not much difference between the RSW- and ice-stored fish. Initially the fish skins had 4 μg/cm² (as lutein) and after 7 days they were approximately 3 μg/cm². It was noted visually that some of the extracts were more yellow and less pink, presumably due to modifications of the carotenoids during storage. Thus, both reflected color and pigment concentration results show at least as good color retention in the ocean perch skins in RSW as in ice. Most of the apparent color differences between the several runs were presumably due to the raw material variations in the fish and in the handling before arrival at the laboratory.

In one experiment, which is representative of the others (Fig. 4, upper curves) ash contents of the fish in both RSW tanks increased whereas that of the fish in ice decreased. As the moisture contents of the fillets did not show significant change during

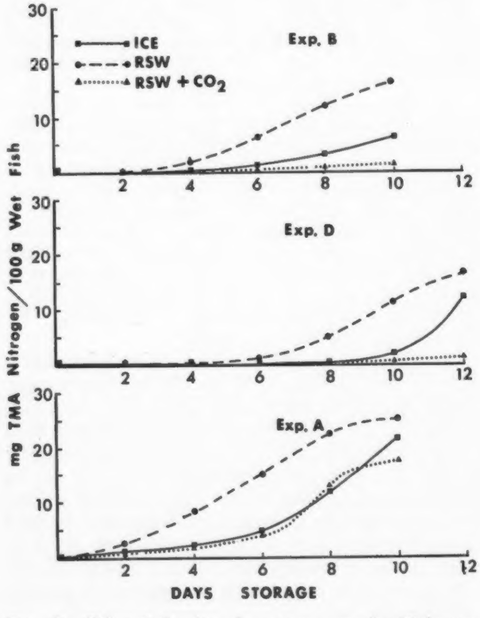

FIG. 2. Trimethylamine in ocean perch (*Sebastes marinus*) fillets from three experiments in which the fish were stored at -1 C in refrigerated sea water or in crushed ice.

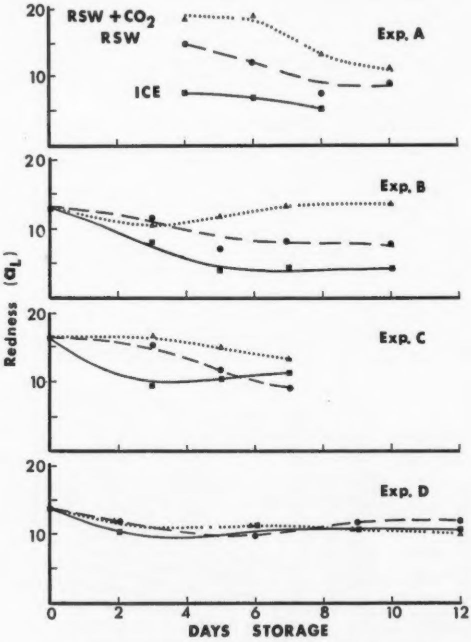

FIG. 3. Redness (a_L) of ocean perch fillet skins from four experiments in which the fish were stored in refrigerated sea water or crushed ice.

storage (78.8–80.2% range) the changes in ash content are mainly related to the uptake or loss of salts as is shown below.

Potassium losses from RSW- and ice-stored fish were about the same as is evident in the typical graph (Fig. 4, middle curves). Up to one third of the potassium was lost from the fillets within the 12-day experimental period.

The diffusion of sodium into the fish became apparent after a few days' storage in RSW (Fig. 4, lower curves). In this typical experiment the initial sodium content was 165 mg/100 g fillet. The fish had clearly not equilibrated with the sea water by the time the experimental run was terminated. The iced fish slowly lost sodium, the loss being over one third of the initial fillet concentration by the end of the experiment.

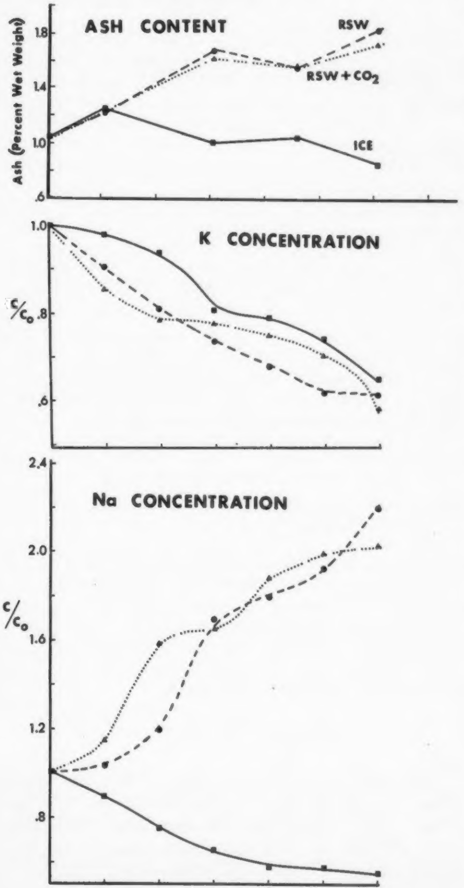

FIG. 4. Ash, potassium, and sodium concentrations in skinned ocean perch fillets from fish stored in refrigerated sea water at –1 C or in ice. (Experimental run D). Potassium and sodium values as ratios of concentrations (C) to initial concentrations (Co; 3360 ppm K and 1650 ppm Na).

Although taste panel evaluations were not done, we had noted previously that the salt level in cod stored in RSW for 5 days was organoleptically noticeable (unpublished data). This was not objectionable; in fact, the flavor was enhanced. As ocean perch, after the same length of time, had approximately the same concentration (250 mg/100 g) taste assessments would be similar. Also, Barnett et al. (1971) showed taste panel data which indicated a nonobjectionable salty taste in rockfish held in RSW + CO_2 up to 17 days. Harrison and Roach (1953) also referred to the salt content as it affected taste and set the level of palatability at about 1% (equivalent to 400 mg sodium/100 g). The salt uptake in the experiments with ocean perch being reported here may be exaggerated due to the 1:1 fish to water ratio. It is expected that higher ratios would be used commercially.

The rate of salt uptake could be drastically reduced if temperatures were lowered enough to freeze the tissues, as has been done with fortified salt water in tuna and salmon industries (Roach et al. 1967). However, the freezing in fortified sea water would introduce added costs for salt, for the greater refrigeration capacity necessary to remove the heat of fusion, and for thawing before processing.

The operational and labor-saving potentials of refrigerated seawater storage of ocean perch can be realized, for the RSW gives at least as good color retention as ice storage and the addition of CO_2 enhances skin color retention and flesh quality retention.

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Factors Mediating Interspecific Aggregation of Tubificid Oligochaetes

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BRINKHURST, R. O. 1974. Factors mediating interspecific aggregation of tubificid oligochaetes. J. Fish. Res. Board Can. 31: 460–462.

Tubifex tubifex and *Limnodrilus hoffmeisteri* are both attracted to the feces of *T. tubifex* rather than to those of *L. hoffmeisteri* or a control sediment. This substantiates the hypothesis that lowered respiration and increased growth caused by mixing the two species could be due to an increase in time spent burrowing, rather than feeding, when isolated species are removed from their preferred food, the feces of another species of worm. It also helps explain the inter- and intraspecific clumping that characterizes their normal distribution.

BRINKHURST, R. O. 1974. Factors mediating interspecific aggregation of tubificid oligochaetes. J. Fish. Res. Board Can. 31: 460–462.

Tubifex tubifex et *Limnodrilus hoffmeisteri* sont tous deux attirés vers les fèces de *T. tubifex* plutôt que vers celles de *L. hoffmeisteri* ou vers un sédiment témoin. Ceci justifie l'hypothèse que l'abaissement de la respiration et l'accroissement de la croissance causés par le mélange des deux espèces seraient le résultat d'un temps plus long consacré au fouissage plutôt qu'à l'alimentation, lorsque les espèces isolées sont éloignées de leur nourriture préférée, les excréments d'une autre espèce de ver. Ceci peut également aider à expliquer l'agglutination inter- et intraspécifique qui caractérise leur distribution normale.

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THE reduction in respiration and increase in feeding activity of tubificid worms in mixed culture (Brinkhurst et al. 1972) could be attributed to a change in behavior dependent on feeding preferences. Tubificids as a group ingest particles selectively (Brinkhurst et al. 1972; Poddubnaja 1961) and three tubificid species fed on the same food source egest different proportions of at least one representative fraction of the bacterial flora present, the heterotrophic aerobes (Brinkhurst and Chua 1969; Wavre and Brinkhurst 1971). Not only that but, under artificial conditions that isolate the worms from any food beyond that already in their gut, after 7 or 9 days the sole remaining heterotroph was different in each tubificid species (apart from experiments in which an *Aeromonas* replaced the normal survivor in any of the worm species used). As 72% of the bacteria were destroyed on passage through the gut, they are likely to represent the primary food source of these sediment-ingesting organisms. Intense food specialization could account for the survival of multispecific aggregations, and this could be mediated through selective ingestion and/or digestion. If the feces of one worm

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species contain a high proportion of bacteria that are the preferred food of another species, aggregations of worms may form by mutual attraction. Solitary species may exhaust their preferred food and may actively burrow in search of preferred food, hence raising their respiration loss, reducing time available for feeding, and lowering their assimilation efficiency by feeding on available food. Mixed species may move around less, feed more, and ingest preferred food. A simple test of this hypothesis is examined here.

Methods and results — Sediments and tubificids (*T. tubifex* and *L. hoffmeisteri*) were collected from the St. John River at Beechwood Dam, N.B. Feces were collected from pure cultures of each species, and small quantities were placed in 15-cm petri dishes, together with a quantity of sand and sterile river mud such that worms placed in the middle of the dish would have three alternative heaps of sediment to crawl into. In the first set of experiments the piles of sediment dried out overnight by mistake, and were reinoculated the next morning. In the second series the sediment was kept moist, but in the third series it was allowed to dry out again, as it was convenient to do this and no significant effect had been detected in the earlier comparison.

Ten worms of one or the other species were placed in the center of each dish equidistant from the sediment piles. The arrangement of the piles was randomized (*Tubifex* – *Limnodrilus* – Control, *Tubifex* – Control – *Limnodrilus* being the only two possible arrangements) as was their orientation on the bench. The whole array of dishes (15 or 20 per trial) was covered with a sheet of black plastic for 24 h, after which the number of worms found in the *T. tubifex* feces (T) or in the *L. hoffmeisteri* feces (L) or in the control (C) was recorded. In all, 30 dishes containing *L. hoffmeisteri* were used but only 25 using *T. tubifex*. The numbers obtained were transformed ($\sqrt{x + 0.5}$) to remove zero records, and the results were analyzed by one-way analysis of variance (Snedecor and Cochran 1968). The transformed means for all data combined were:

	Test sediment			
	C	T	L	N
T. tubifex	2.05	4.74	2.47	25
L. hoffmeisteri	1.21	5.54	2.34	30

L. hoffmeisteri was attracted to the feces of *T. tubifex* ($F = 18.03$, $P < 0.01$) but *T. tubifex* was also attracted to its own feces ($F = 6.68$, $P < 0.01$).

Independent evidence to support the contention that *L. hoffmeisteri* is attracted to *T. tubifex* was obtained in another set of experiments designed to test the attraction to both the live worm and its products. In this arrangement, water passing over *Tubifex* and *Limnodrilus* cultures drained into opposite ends of an elongate third container with a central overflow. About 100 *L. hoffmeisteri* were released near the center of the third container and were allowed to distribute themselves freely. After 5 h the worms were recovered and allocated to one of five equal zones in the container (below the *Tubifex* culture overflow, between that and the central zone, around the central zone, between center and *Limnodrilus* overflow, and beneath the latter — designated zones A–E). Three trials were run with this array set up one way on the bench (*T. tubifex* culture to the right) and two the opposite way around in case there was any subtle environmental gradient in the laboratory. The results were:

	Zones of test vessel				
Orientation	A	B	C	D	E
1	3	26	91	2	3
	16	27	50	10	3
	0	4	90	14	4
2	2	9	97	3	1
	6	14	76	7	2
Σ	27	80	404	36	13
	107		404	49	

In all experiments most worms remained close to the point at which they were introduced but in all but one experiment and certainly on average, the rest of the *L. hoffmeisteri* tended to be displaced toward the *Tubifex* overflow rather than to that derived from their own species. No statistical analyses of the data have been carried out and it is mentioned here only as independent support to the results from the first simple experiment.

Discussion — These results strongly support the hypothesis outlined in the introduction, providing a laboratory demonstration that at least one tubificid species can and will move in search of the feces of another species and hence a functional explanation of the observed microdistribution of these organisms in natural sediment. Most accounts of interspecific interaction yield explanations for coexistence based on niche specialization, food refugia, and spatial or temporal separation of microhabitats and as such emphasize negative interactions. The simple experiments described above make it possible to link laboratory-observed positive interactions between free-living invertebrates with known distribution patterns in the field. This suggests that such positive interactions may well occur in nature, providing an unusual (if not unique) example of mutualism between free-living organisms belonging to a single family.

Positive interspecific associations are well known in the spectrum of commensalism, mutualism, and symbiosis, but they usually involve pairs of species with no close systematic relationship. Interspecific dispersion of territorial birds (Wynne-Edwards 1962) may involve closely related species and is geared to proper utilization of available resources, though in that instance it ensures the exclusion of a potential competitor rather than the subdivision of the resource. Birds that share colonies and mixed ungulate herds may often share a common food source, or compete for it, but it is probable that studies of these interactions are not precise enough to reveal all of the finer specializations that permit survival without competitive exclusion. In fact, the difficulty with identifying exceptions to that principle is that one cannot marshal positive evidence of a lack of specialization.

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Effect of Complexation on Toxicity of Copper to Fishes

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Copper (Cu) is highly complexed by carbonate and hydroxide ions in natural waters and this complexation determines the concentration of copper species in solution. Results of detailed equilibrium calculations on data from bioassays where alkalinity, *p*H, hardness, and total copper concentration are different indicate that copper(II) is the chemical species that is toxic to fishes and that alkalinity is the factor controlling copper(II) concentration.

PAGENKOPF, G. K., R. C. RUSSO, AND R. V. THURSTON. 1974. Effect of complexation on toxicity of copper to fishes. J. Fish. Res. Board Can. 31: 462–465.

Le cuivre (Cu) est fortement complexé par les ions carbonate et hydroxyde dans les eaux naturelles, et cette complexation détermine la concentration de l'espèce de cuivre en solution. Les résultats de calculs détaillés d'équilibre sur des données provenant d'analyses biologiques dans lesquelles on fait varier l'alcalinité, le *p*H, la dureté et la teneur en cuivre total indiquent que le cuivre(II) est l'espèce chimique qui est toxique pour les poissons et que l'alcalinité est le facteur qui contrôle la teneur en cuivre(II).

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TOXICITY of copper to fishes has been the subject of many research papers, some of which have been reviewed by Doudoroff and Katz (1953) and McKee and Wolf (1963). A number of these papers have addressed the variables that have an effect upon the toxicity, such as water hardness, temperature, dissolved oxygen, and *p*H. Of these factors hardness was considered to be of primary importance (Lloyd 1965). Stiff (1971), using the equilibrium equations of Scaife (1957), investigated the formation of complex carbonate species in copper–bicarbonate solutions and suggested that the difference in toxicity of copper to fish with differences in water hardness might be dependent upon carbonate complexation.

In this report we present an equilibrium model and its application to several literature bioassay test systems. The model involves detailed equilibrium calculations that consider hardness and complexation by both inorganic carbon and hydroxide. The results of this study indicate that Cu^{2+} is a toxic species. $CuOH^+$ may also be toxic.

Methods — The chemical equilibria considered in our calculations are presented in Table 1. Most of the constants are for $\mu \longrightarrow 0$ and T = 20 C. The experimental parameters utilized in our calculations were *p*H, total copper concentration, alkalinity, and hardness. Many reports in the literature could not be used because they do not include all these necessary parameters.

Chelating agents such as nitrilotriacetic acid, citric acid, and humic acid may reduce copper toxicity (Sprague 1968; Nishikawa and Tabata 1969; Wildish et al. 1971). No organics were reported in the test waters of the bioassays we considered in this study, and we assumed they were not present in significant amounts. Consequently, we omitted them from our model.

Solution of the simultaneous equilibria presented in Table 1 was achieved by computer, using the program "COMICS" (Perrin and Sayce 1967) which was adapted to the MSU Sigma 7. The input parameters are *p*H, total copper concentration, total inorganic carbon, and total calcium. Small changes in the values of the equilibrium constants will have little effect on the calculated species distribution as the *p*H values are reported to only one significant figure. Total copper concentration was used as reported. Total inorganic carbon was calculated from alkalinity and *p*H using the following equation:

$$\text{inorganic carbon} = \left[\frac{\dfrac{\text{alkalinity}}{10^{-6.36}}}{10^{-pH} + 10^{-6.36}}\right]$$

This is a valid operation as long as no significant amounts of weak acids such as hydrogen phosphate and humic acid are present. Literature values for hardness are reported as milligrams per liter $CaCO_3$ with no differentiation between calcium(II) and magnesium(II). We have set the total calcium concentration equal to the hardness. This is a reasonable assumption because the equilibrium constants for calcium(II) and magnesium(II) are almost the same (Sillen and Martell 1964).

Results and discussion — To be incorporated into this study, the literature reports had to provide sufficient chemical data for use in our calculations. For comparative purposes it was necessary to obtain bioassay results from tests conducted on the same species of fish for comparable periods of time, and the test waters for these bioassays had to be of different hardnesses and alkalinities. Our search of the literature on copper toxicity revealed four papers (Mount 1968; Mount and Stephan 1969; Pickering and Henderson 1966; Lloyd 1961) which fulfilled the above conditions. These reported data for four bioassays on fathead minnows (*Pimephales promelas*) and two bioassays on rainbow trout

TABLE 1. Equilibria for copper test water solutions.

Reaction	log K_{eq}	Reference
$H^+ + OH^- = H_2O$	14.00	Sillen and Martell (1964)
$H^+ + CO_3^{2-} = HCO_3^-$	10.30	"
$2H^+ + CO_3^{2-} = H_2CO_3$	16.76	"
$Ca^{2+} + CO_3^{2-} = CaCO_3$	3.2	"
$Ca^{2+} + H^+ + CO_3^{2-} = CaHCO_3^+$	11.6	"
$Cu^{2+} + CO_3^{2-} = CuCO_3$	6.77	Silman (1958)
$Cu^{2+} + 2CO_3^{2-} = Cu(CO_3)_2^{2-}$	10.01	Silman (1958)
$Cu^{2+} + H_2O = CuOH^+ + H^+$	-7.53	Owen and Gurry (1938)
$2Cu^{2+} + 2H_2O = Cu_2(OH)_2^{2+} + 2H^+$	-10.53	Perrin (1960)

(*Salmo gairdneri*). The data utilized from these studies are listed in the first four columns of Table 2. The value for the total copper concentration in test F was taken from a graph and thus is subject to some uncertainty. The data are for both static and flow-through bioassays, as indicated in Table 2. Our derived values for inorganic carbon and the equilibrium concentrations of copper species resulting from our calculations also are presented in Table 2.

Every natural water system that contains inorganic carbon possesses the capacity to complex copper ions. The degree of complexation increases with increasing alkalinity and *p*H. As a consequence those solutions with high alkalinity and a *p*H of 8 or higher will be able to complex a high percentage of the total copper present. Copper also forms two soluble hydroxide species. The formation of these is in competition with the complexing of copper by inorganic carbon.

Previous experimental studies have shown in general that fish have a greater copper tolerance in hard water than soft; however, an increase in the hardness of a water is accompanied by an increase in alkalinity and thus the complexing capability.

Detailed species distribution for copper in test waters of different alkalinities has been presented in Table 2. There is a large range of total copper concentration and of alkalinity and therefore a large variation in the concentrations of all but one of the resultant copper species. If one of the copper species is responsible for the toxicity, its concentration should be nearly constant for all the studies.

For the fathead minnow data, examination of the calculated equilibrium concentrations (Table 2) of the five possible copper species shows that the concentrations of $CuCO_3$, $Cu(CO_3)_2^{2-}$, $Cu_2(OH)_2^{2+}$, and $CuOH^+$ vary by approximately one to two orders of magnitude. Because of these variations in concentration, none of these species is thought to be the controlling toxic agent. The concentration of Cu^{2+}, however, remains nearly constant from test to test ($11–42 \times 10^{-8}$ *F*) and therefore it is concluded to be the major toxic species. The average Cu^{2+} concentration is $22 \pm 12 \times 10^{-8}$ *F*. When one considers the variation in total copper (3.8×10^{-7}–230×10^{-7} *F*), this agreement seems to be very good. $CuOH^+$, with an eightfold variation in concentration may also be toxic, but less so than Cu^{2+}. In every case a sizable fraction of the total copper is complexed by both carbonate and hydroxide.

The same considerations apply to the rainbow trout studies. Only the concentration of copper(II) remains nearly constant. Again, the evidence indicates that copper(II) is the major toxic species.

Another possible species that may form in natural water is $CuHCO_3^+$. We have done the calculations including this species using an estimated stability constant of $\log K = 12.3$ (Childs 1971). The inclusion of this species results in a less than 1% change in the concentrations of the other five copper species. The concentration of the species varies by more than a factor of 10 and thus its inclusion is not critical.

Calcium and magnesium can influence the copper toxicity indirectly through complexation of carbonate and bicarbonate. The more carbonate and

TABLE 2. Copper species distribution and fish toxicity.

Test no.	Cu_T[a] $\times 10^7$	Alkalinity $\times 10^4$	*p*H	Hardness $\times 10^4$	Inorganic carbon $\times 10^4$	$CuCO_3$ $\times 10^6$	$Cu(CO_3)_2^{2-}$ $\times 10^8$	$Cu_2(OH)_2^{2+}$ $\times 10^{10}$	$CuOH^+$ $\times 10^8$	Cu^{2+} $\times 10^8$
					Fathead minnow					
A[b]	74.0	16.1	7.9	19.8	16.5	6.68	6.97	66.7	44.4	18.9
B[b]	11.8	3.00	7.2	3.14	3.43	0.56	0.022	13.0	19.6	41.8
C[b]	229	30.0	8.2	36.0	30.4	21.1	77.6	213	79.4	17.0
D[b]	3.78	1.80	7.5	2.00	1.93	0.17	0.008	3.29	9.85	10.6
					Rainbow trout					
E[b]	173	24.0	7.7	32.0	25.1	15.9	15.3	177	72.2	48.8
F[b]	7.95	–	6.5	1.75	1.37	0.04	0.001	1.39	6.39	68.5

[a] All concentrations are in formal units. Cu_T refers to total copper concentration.

[b] A, Mount (1968), 96-h flow-through test, fish size 1.0–1.5 cm; B, Mount and Stephan (1969), 96-h flow-through test, fish size 1.0–2.0 cm; C, Pickering and Henderson (1966), 96-h static test, fish size 4–7 cm; D, Pickering and Henderson (1966), 96-h static test, fish size 1.5–2.5 cm; E, Lloyd (1961), 72-h static test, fish size 7.5 cm; F, Lloyd (1961), 72-h static test, fish size 7.5 cm.

bicarbonate complexed by calcium and magnesium, the less that will be available for complexation with copper; this in turn will raise the copper(II) concentration in solution. However, the degree to which calcium and magnesium can raise the free copper concentration is small. This is primarily because in the water systems of the bioassays discussed here there is a very small percentage of the calcium complexed even at high alkalinity and hardness. As a consequence, alkalinity and not water hardness is the major factor that controls copper concentration.

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Effects of the Insecticide Carbaryl on Clams and Some Other Intertidal Mud Flat Animals[1]

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Armstrong, D. A., and R. E. Millemann. 1974. Effects of the insecticide carbaryl on clams and some other intertidal mud flat animals. J. Fish. Res. Board Can. 31: 466–469.

The insecticide carbaryl (Sevin) significantly reduced numbers of juvenile clams in plots treated with 2.3 and 4.6 kg (5 and 10 lb)/acre. Pooling of samples taken at 1, 2, 4, 15, and 30 days after treatment showed mean clam numbers per m² in untreated and treated plots were 364, 283, and 224, respectively, the reductions from the controls being 22 and 38%. Clam species differed in susceptibility to carbaryl; for example, numbers of the gaper clam (*Tresus capax*) were reduced by 58 and 69% at the low and high application rates in relation to those from the control plots, and the bent-nosed clam (*Macoma nasuta*) by 9 and 28%. There was no reduction in numbers of polychaete and nemertean worms.

A carbaryl application of 2.3 kg/acre was as effective in controlling ghost shrimp (*Callianassa californiensis*), an oyster pest, as 4.6 kg/acre. Signs of stress and numbers affected are given for other mud flat animals.

Armstrong, D. A., and R. E. Millemann. 1974. Effects of the insecticide carbaryl on clams and some other intertidal mud flat animals. J. Fish. Res. Board Can. 31: 466–469.

L'insecticide carbaryl (Sevin) a réduit de façon significative le nombre de jeunes coques dans des lots traités à des concentrations de 2.3 kg et 4.6 kg (5 et 10 lb) à l'acre. La mise en commun des échantillons prélevés à intervalles de 1, 2, 4, 15 et 30 jours après traitement démontre que le nombre moyen de coques par m² dans les lots non traités et traités est de 364, 283 et 224 respectivement, la réduction chez les témoins étant de 22 et 38%. Les différentes espèces de coques ont une susceptibilité différente au carbaryl; c'est ainsi, par exemple, que le nombre de la coque *Tresus capax* diminue de 58 et 69% aux taux bas et élevé d'application, comparativement à celui des lots témoins; la coque *Macoma nasuta* diminue de 9 et 28%. Il n'y a pas de diminution du nombre de vers polychètes et némertiens.

Une application de carbaryl à raison de 2.3 kg/acre contrôle aussi efficacement la callianasse (*Callianassa californiensis*), une pest de l'huître, qu'une application de 4.6 kg/acre. Nous décrivons les signes de stress et indiquons les nombres affectés chez les autres animaux de plage de vase.

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Carbaryl (Sevin; 1-naphthyl methylcarbamate) has been used in bays and estuaries to control oyster pests and predators on the Atlantic coast (Loosanoff 1960) and in the Pacific Northwest (Snow and Stewart 1963; Washington Department of Fisheries 1970). There have been only two reported field studies of carbaryl's toxicity to oyster pests and nontarget animals (Snow and Stewart 1963; Washington Department of Fisheries 1970), and in neither study were quantitative effects of treatment on indigenous clams given. This note reports on field studies conducted to determine: (1) whether application of carbaryl (Sevin; 80% wettable powder) to mud flats would reduce the numbers of juvenile clams and other invertebrates; and (2) whether reduction, if any, would be greater at the standard rate of application of 4.6 kg (10 lb)/acre than at half this amount. Also, a more limited study was conducted to determine if 2.3 kg (5 lb)/acre was as effective as the higher rate in controlling the ghost shrimp, *Callianassa californiensis*, which is one cause for oyster ground rehabilitation in the Pacific Northwest.

Materials and methods — To compare effects of the rates on juvenile clams and other invertebrates, six

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plots 3.7 m^2 (12 ft) were established in the Sally's Bend area of Yaquina Bay, Oregon. Toward the middle of each plot, five 50- × 150-cm subplots adjacent to each other were marked with stakes and rope. To minimize variation in substrate type and resident animal species, the two plots to be treated at each of the two rates were located 4.6 m (15 ft) apart, and two untreated plots were 9.1 m (30 ft) from these. Plots were located at a 0.0-m tidal level in a line at right angles to the flow of ebb and flood tides, to help reduce the transport of residual carbaryl from treated to control plots.

The insecticide was applied by passing over the plots a perforated bucket containing a suspension of carbaryl in sea water until they were covered uniformly. Plots were treated on May 14, 1972 during low tide and remained uncovered, except for water left in depressions, for 2.5 h. The temperature of the water remaining on the plots was 16 C. The concentration of carbaryl in this water immediately after treatment was not determined. However, since the solubility of carbaryl in sea water is 60 mg/liter at 20 C (Dr D. Coulsomb, Stanford University, Stanford, Calif., personal communication) the carbaryl concentration in the water on the plots and in the animal burrows must have been less than this value.

An entire subplot in each of the six plots was sampled on days 1, 2, 4, 15, and 30 after treatment, the total area sampled for each type of treatment being 7.5 m^2. In sampling, all mud was removed to a depth of 12.7 cm (5 inches), thus retrieving most juvenile clams of the 0-yr age-group. Samples were washed over a screen of 1.5-mm mesh in the field, and the animals were sorted and numbers of each species recorded in the laboratory. The data were subjected to two-way analysis of variance (Snedecor and Cochran 1967) to determine the effect of treatment on the survival of animals to 30 days at the two application rates.

The Japanese littleneck clam, *Venerupis japonica*, was introduced onto all plots 1 day before treatment. The effect of carbaryl on this clam's survival was of interest because of its recent introduction into Oregon bays. Eighty clams were placed on each subplot and observation of the animals after planting confirmed that they all burrowed into the substrate.

To compare the effectiveness of the two rates of application on controlling ghost shrimp, three 3.7 m^2 plots were established in an area of Yaquina Bay that is heavily infested with *C. californiensis.* On July 9, 1971, two plots were treated at 2.3 and 4.6 kg/acre, and the remaining plot was the untreated control. In June 1972, these plots were examined for recolonization by ghost shrimp. Shrimp densities were estimated by counting their burrows in randomly selected areas of about 0.1 m^2 (1 ft^2) each.

Sample analyses — There were significant reductions ($\alpha = 0.01$) in the total numbers of the five common species of clams on the treated plots (Table 1). The mean numbers of these animals per m^2 in the 2.3 and 4.6 kg/acre plots were 22 and 38% less ($\alpha = 0.01$) than those in the untreated plots. The difference between the two rates of application (21%) was significant ($\alpha = 0.01$). Also, the inverse relationship between the rates of application and the numbers of clams surviving was consistent for the five samplings (Fig. 1).

The smaller numbers of animals (Fig. 1) in the six subsamples taken on day 30 than on the 4 previous days for the same plots, may be attributed to the order in which subplots were sampled. Those for day 30 were between subplots previously excavated, so that the sampling areas were elevated strips subject to tidal action, and some of the clams may have been lost by erosion and predation.

Analysis of variance of the data on the interaction of time with treatment for single species showed that significance for this interaction occurred only with the gaper clam (Tresus capax). Thus, levels of carbaryl lethal to the other clams did not persist, and most of the mortalities occurred within the first 24 h. There was no apparent reduction in numbers of remaining animals in the samples, polychaete worms, and a few nemertean worms.

The lower rate was as effective as the higher in controlling ghost shrimp. After 11 mo, the mean numbers of shrimp holes/m^2 were 161, 32, and 25 in the control plot and 2.3 and 4.6 kg/acre plots. The difference between the control plot and either of the two treated plots was significant ($\alpha = 0.01$), but there was no significant difference between the two treated plots.

Field observations — As application rates exceeded the water solubility of the pesticide, the inert carrier and undissolved carbaryl were visible as a white layer that almost obscured the mud. There was no indication of transport of undissolved carbaryl beyond the plots before the tide returned. But dissolved carbaryl obviously was moved away from the treated plots by subterranean seepage as the tide receded, because dead shrimp, *Crago nigricauda*, and English sole, *Parophrys vetulus*, were found as far as 15 m from the treated areas in the direction of tidal recession. We could not determine the extent of pesticide transport by subsurface routes. The material in the animal burrows could be moved through the tunnel network in the water by resident animals. From MacGinitie's description of shrimp tunnels (1930, 1934) carbaryl may have been transported as much as 76 cm vertically and about 1 m horizontally in the extensive burrow network. The pesticide on the surface or in the burrows probably did not penetrate more than 10 cm into the surrounding mud, because Karinen et al. (1967) found very low levels of carbaryl beyond this depth when it was applied to a mud substrate.

No dead or distressed animals were observed on or in the immediate vicinity of control plots. The only animals to die on plots after treatment and before the first flood tide were about 200 opisthobranchs, *Ogleija diomedea*, and several fish, and the numbers of dead animals on plots treated with the two rates were similar. Most moribund and dead animals at this time were several meters from treated plots in beds of eel grass, *Zostera marina.* Animals began to die in, on, and near

TABLE 1. Mean numbers of various clams and worms in duplicate 0.75 m^2 areas of mud, sampled on five occasions during 30 days, from field plots treated with carbaryl at 0.0, 2.3, and 4.6 kg/acre.

Species	Carbaryl concentration (kg/acre)		
	0	2.3	4.6
Clam			
Macoma nasuta (bent-nosed clam)	350	317(9)[a]	253(28)
M. incongrua (Incongruous *Macoma* clam)	31	26(16)	17(44)
M. inconspicua (Inconspicuous *Macoma* clam)	76	49(36)	39(49)
Tresus capax (gaper clam)	60	25(58)	19(69)
Venerupis japonica (Japanese littleneck clam)	37	18(52)	15(61)
Subtotal	554	435(22)[b]	343(38)[b]
Number per m^2	365	283[b]	224[b]
Transenella tantilla	5	2	2
Psephidia lordi	1	1	1
Clinocardium nuttalli (basket cockle clam)	2	0.4	1
Venerupis staminea (Pacific littleneck clam)	1.6	0.2	0.2
Mya arenaria (soft-shelled clam)	1	0.4	0.4
Polychaetes			
Nephtys sp.	3.2	3.4	3.0
Haploscoloplos elongatus	35	29.6	31.2
Goniana brunnea	13	12	13
Spionid sp.	2	5.6	4
Nemerteans			
Cerebratulus sp.	1	1	1
Totals	618	490[b]	399[b]

[a] % fewer than in control plots.
[b] Differences between control and treated plots and between treated plots significant at $\alpha = 0.01$.

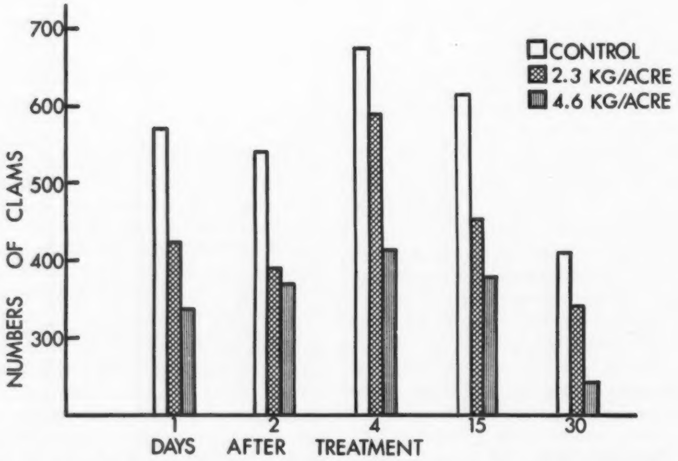

FIG. 1. Effects of the insecticide carbaryl on numbers of clams remaining alive in field plots sprayed with the chemical at 2.3 and 4.6 kg (5 and 10 lb)/acre.

the treated plots within 5 min after application of carbaryl. Usually, the first stress sign in some fish and crustaceans was hyperactivity in the form of rapid and erratic darting movements through the shallow water. This was followed by body rigidity, paralysis, and death. Eighty English sole and over 1000 *Crago* shrimp were counted dead. Most of these animals were observed and counted during hyperactivity, as their protective coloration effectively concealed them when quiescent. Therefore, the observed numbers of affected animals may be considerably less than the true numbers.

The only clams to exhibit stress signs initially following application of carbaryl were adult basket cockles, *Clinocardium nuttalli*, which emerged from the mud, opened their valves, and made digging movements with their feet. No juvenile clams of any species were seen leaving the mud. Many feeding clams, *Macoma* spp., in the standing water were observed taking in sediment, including the particulate carbaryl. In laboratory studies (unpublished data) with adult bent-nosed clams, *M. nasuta*, we found that the animals in carbaryl concentrations as high as 25 mg/liter initially behaved normally. The siphons were extended and food-searching movements continued, but eventually the clams became paralyzed and could not retract their siphons even when mechanically stimulated.

One day after treatment, there were 52 dead Dungeness crabs (*Cancer magister*) near the plots and 30 had been partially eaten by gulls. There were live English sole on and around all plots indicating the toxic levels of carbaryl for these fish must have decreased considerably in 24 h.

Discussion — The results of our study provide the first demonstration that juvenile clams are significantly reduced in numbers by application of carbaryl to estuarine mud flats. Loosanoff et al. (1960), Haydock (1964), and Haven et al. (1966), using carbaryl in conjunction with Polystream, noted adverse but not always lethal effects of carbaryl on gastropods such as distention of the foot, and Lindsay (1961) and Chambers (1970) reported deaths of clams after treatment of a mud flat with carbaryl, but the numbers of dead animals were not determined in either study.

Though Stewart et al. (1967) and Butler et al. (1968) found in laboratory studies that 1-naphthol, the first hydrolytic product of carbaryl, was more toxic to mollusks than the parent compound, 1-naphthol appeared to have little if any toxic effect on the animals in our study. The first returning tide diluted and removed most of the carbaryl from the surface of the mud flats. Karinen et al. (1967) reported that only 3.3 to 0.46 ppm of carbaryl remained in the top 1 and 3 inches of mud 24 h after it was sprayed, in comparison with 0.30 ppm to undetectable levels of 1-naphthol, indicating slow hydrolysis of carbaryl in mud. Butler et al. (1968) reported that no juvenile basket cockle clams died during a 24-h laboratory exposure to 5.60 mg/liter of 1-naphthol, a concentration 18 times higher than that found by Karinen et al. (1967) in treated mud. Finally, we found that significant effects of treatment occurred within 24 h after application and very little 1-naphthol would have been formed by this time.

Our finding that carbaryl did not reduce the number of polychaete or nemertean worms differs from those of Snow and Stewart (1963) and Chambers (1970), who reported deaths and a decline in numbers of annelid worms after treatment of mud flats with carbaryl. In this study, the fine particle size of mud flat substrate that resulted in poor interstitial circulation, and the removal of carbaryl by the first returning tide, probably prevented toxic levels from reaching most of the worms.

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An Infestation of the Marine Fish *Lycodes lavalaei* by the Parasitic Copepod *Tanypleurus alcicornis*

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KHAN, R. A., AND T. K. PITT. 1974. An infestation of the marine fish *Lycodes lavalaei* by the parasitic copepod *Tanypleurus alcicornis*. J. Fish. Res. Board Can. 31: 470–471.

Three eelpouts, *Lycodes lavalaei*, with gills infested by the parasitic copepod *Tanypleurus alcicornis* (Caligoida: Tanypluridae) were collected on the Grand Banks off Newfoundland. As many as 42 parasites were recorded from one fish. The copepods caused extensive damage to the gill filaments. Anemia and emaciation were apparent in the infested fish.

KHAN, R. A., AND T. K. PITT. 1974. An infestation of the marine fish *Lycodes lavalaei* by the parasitic copepod *Tanypleurus alcicornis*. J. Fish. Res. Board Can. 31: 470–471.

Trois lycodes, *Lycodes lavalaei*, ont été capturées sur les Grands bancs, au large de Terre-Neuve, dont les branchies étaient infestées par le copépode parasite *Tanypleurus alcicornis* (Caligoida: Tanypluridae). Nous avons compté jusqu'à 42 parasites chez un poisson. Les copépodes avaient causé des dommages considérables aux filaments branchiaux. Les poissons infestés souffraient manifestement d'anémie et d'émaciation.

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AN eelpout, *Lycodes lavalaei* Vladykov and Tremblay, with gills infested by a parasitic copepod, was collected in May 1972 on the Grand Banks off Newfoundland (48°05′N, 50°14′W) from 220 m. In April 1973, two additional eelpouts were collected on the Banks (45°58′N, 52°39′W) from 108 m and both were also heavily infested with the same copepod. Dissection and examination of the parasites revealed that they agreed with Kabata's (1969) description of *Tanypleurus alcicornis* (Caligoida: Tanypleuridae) and are so identified herein.

The parasites were attached to the dorsal surfaces of the branchial arches, and either destroyed and lay in the place of or displaced the gill filaments. In one fish, most of the gill filaments in the right branchial chamber as well as those of three gill arches on the left side were absent (Fig. 1, 2). A total of 42 parasites were recorded from this

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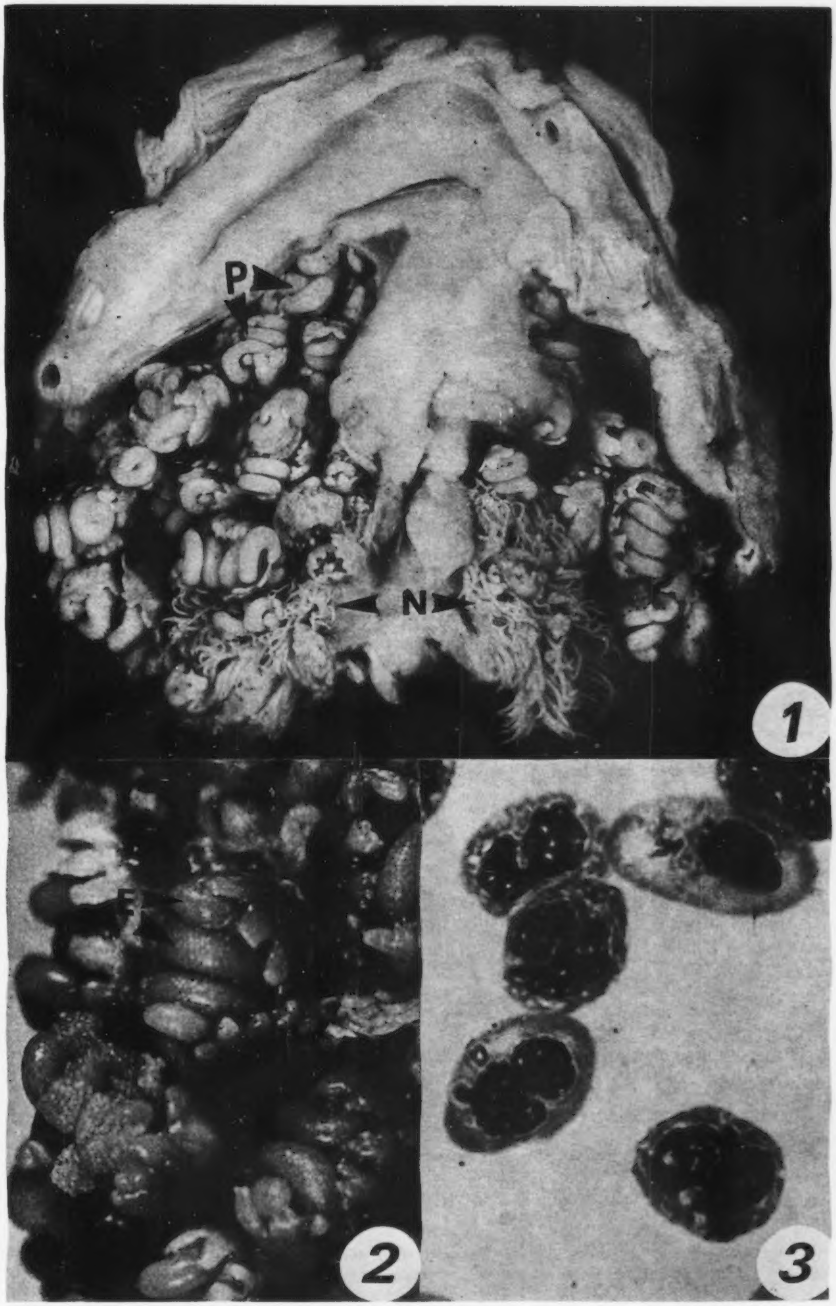

FIGURES 1–3.

FIG. 1. Lower jaw with gills intact showing infestation with *Tanypleurus alcicornis*. × 2. P, *parasite; N, normal gills;* FIG. 2. *Close-up view of parasite showing egg sacs (E).* × 4; FIG. 3. Cardiac blood smear. Note segmented nuclei of erythrocytic precursors. × 2400.

Khan and Pitt — J. Fish. Res. Board Can.

fish and the numbers were only slightly fewer in the other two infested animals.

According to Kabata (1969), *T. alcicornis* was described from the Atlantic spiny lumpsucker, *Eumicrotremus spinosus*, taken off Greenland. Forty-eight specimens of the latter fish from the Grand Banks that we examined during 1972–73 did not harbor any parasitic copepods. Kabata (1969) found the parasite on one of three Arctic eelpouts, *Lycodes reticulatus*. We have, to date, observed it on only three of 62 *L. lavalaei*.

Examination of sections stained with hematoxylon and eosin from the affected region revealed extensive damage of the gill filaments. Most small blood vessels were destroyed and there was evidence of inflammation, with necrotic tissue in some parts, leaving only traces of the normal structure. Some of the gill filaments were replaced by connective tissue. Few normal branchial filaments remained and these were pushed aside by the parasites. The former, almost white in color, suggested severe anemia. The fish appeared extremely emaciated.

An abnormal blood composition was observed in the fish. The hematocrit readings varied from 5 to 11 (normal readings, 18–22) and the hemoglobin content was reduced to 0.6–0.8 g (normal values, 3.0–8.0 g/100 ml). Examination of cardiac blood smears revealed erythrocytic anisocytosis. There was an increase of erythrocytic precursors, the nuclei of which were often segmented (Fig. 3). Normal erythrocytes and erythrocytic precursors occurred in the ratio 16:1000 in one of the animals. Hyperplasia and hypertrophy of erythroid elements were evident in stained impression smears of the kidney. An exhaustive examination of the blood failed to reveal the presence of any blood parasites.

A trichodinid ciliate also parasitized the gills of one of the three fish. Examination of scrapings from normal gill filaments revealed that the infection was heavy.

The severe anemia noted in the three eelpouts was probably attributable to *T. alcicornis* and not to any other parasite, e.g. *Trichodina* sp. Moreover, the presence of the hosts' blood in the copepods suggests that blood loss was probably due to its removal by the parasites rather than other means, e.g. lytic substances inoculated by the copepods during the act of feeding or hemorrhage from ruptured blood vessels of the gill filaments.

Acknowledgments — We are most grateful to Dr Z. Kabata, Department of the Environment, Fisheries and Marine Service, Biological Station, Nanaimo, B.C. for identifying the copepod, and to colleagues for their critical review of the manuscript. The project was supported by funds from the National Research Council of Canada.

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Northern Extension of Known Range of Atlantic Menhaden (*Brevoortia tyrannus*)

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PEPPAR, J. L. 1974. Northern extension of known range of Atlantic menhaden (*Brevoortia tyrannus*). J. Fish. Res. Board Can. 31: 471–472.

Ten specimens of Atlantic menhaden (*Brevoortia tyrannus*) were taken in salmon trapnets near Dalhousie, New Brunswick, from June 18 to July 19 in 1973. The northern limit of range of the species was previously considered to be western Nova Scotia.

PEPPAR, J. L. 1974. Northern extension of known range of Atlantic menhaden (*Brevoortia tyrannus*). J. Fish. Res. Board Can. 21: 471–472.

Dix spécimens d'alose tyran (*Brevoortia tyrannus*) ont été capturés dans des trappes à saumons, près de Dalhousie, Nouveau-Brunswick, entre le 18 juin et le 19 juillet 1973. La limite nord connue jusqu'ici de cette espèce était l'ouest de la Nouvelle-Ecosse.

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TEN specimens of Atlantic menhaden (*Brevoortia tyrannus*) were caught in a floating salmon trapnet in Chaleur Bay, just south of Bon Ami Rocks, Dalhousie, New Brunswick (48°03′N; 66°21′W) in 1973. Nine specimens were captured from June 18 to 28 and the 10th on July 19. Three specimens, two females and a male, were kept for subsequent identification and were preserved in 10% formaldehyde shortly after capture.

Morphometric and meristic data were determined from two preserved females, 301 and 279 mm standard length; scales were removed for age determination. All measurements and counts are according to Hubbs and Lagler (1958).

Data obtained from the two females are within ranges reported for the species by Jordan and Evermann (1896), Bigelow and Schroeder (1953), Hildebrand (1963), and Leim and Scott (1966). Following are measurements and counts for the specimens (301 and 279 mm standard length, respectively): orbit length, 16 and 14 mm; greatest body depth, 98 and 95 mm; depth caudal peduncle, 28 and 27 mm; length of maxillary, 47 and 42 mm; length of dorsal fin base, 63 and 51 mm; length of anal fin base, 45 and 44 mm; length of pectoral fin, 53 and 50 mm; length of pelvic fin, 24 and 23 mm; length of caudal fin, 89 and 82 mm; dorsal fin rays, (1) 19; anal fin rays, 20. The ovaries were in a maturing or developing condition. The male, 285 mm standard length, showed maturing gonads. There were at least four annuli (Hildebrand 1963) on the scales of all three specimens, indicating that these were sexually mature fish (Bigelow and Schroeder 1953).

Leim and Scott (1966) state that the range of Atlantic menhaden is from western Nova Scotia to Florida and that the species usually occurs in Canada only as a stray. Occurrences of the species have been reported from the continental waters of North America as far north as Saint John, N.B. and the lower Bay of Fundy (Leim and Scott 1966). More recently, a single specimen was taken in Kouchibouguac Bay, N.B. in 1970 (Lear and Pippy 1971). The new records are all the more important because of the number of fish involved, and represent a significant northern extension of the known range of the species in the western Atlantic.

The three specimens are in the collection of Environment Canada's Resource Development laboratory facilities at Millbank, New Brunswick.

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Effect of Mincing on Glycolytic Activity in Prerigor Atlantic Cod (*Gadus morhua*) Muscle Stored in Ice or Frozen

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NOWLAN, S. S., AND W. J. DYER. 1974. Effect of mincing on glycolytic activity in prerigor Atlantic cod (*Gadus morhua*) muscle stored in ice or frozen. J. Fish. Res. Board Can. 31: 473–476.

Mincing caused at least a threefold increase in the postmortem rates of both glycogen breakdown and loss of high energy phosphate in unfrozen samples of prerigor Atlantic cod (*Gadus morhua*) muscle stored at 0 C. During freezing and holding at –3 and –6 C these reactions were accelerated, but the rates in the frozen mince were similar to those in the unminced muscle indicating that mincing has no apparent stimulatory effect on glycolytic activity and dephosphorylation of ATP if the muscle is frozen immediately after it is minced. Thus, minced muscle can be used instead of intact slices in glycolytic studies below 0 C with comparable results so long as freezing takes place immediately after mincing. During large-scale preparation of deboned comminuted muscle from prerigor fish, some glycolysis will occur dependent on the time delay and temperature of holding prior to subsequent freezing.

NOWLAN, S. S AND W. J. DYER. 1974. Effect of mincing on glycolytic activity in prerigor Atlantic cod (*Gadus morhua*) muscle stored in ice or frozen. J. Fish. Res. Board Can. 31: 473–476.

La mise en hachis entraîne une augmentation d'au moins le triple dans les taux postmortem à la fois de dégradation du glycogène et de perte de phosphate de haute énergie dans des échantillons non congelés de muscle de morue de l'Atlantique (*Gadus morhua*) prérigor entreposés à 0 C. Ces réactions s'accélèrent pendant la congélation et le maintien à –3 et –6 C, mais les taux dans le hachis congelé sont semblables à ceux du muscle non haché, ce qui indique que la mise en hachis n'a pas d'effet stimulant apparent sur l'activité glycolytique et la déphosphorylation de l'ATP si le muscle est congelé immédiatement après la mise en hachis. Le muscle haché peut donc être utilisé à la place de tranches intactes dans des études de glycolyse à des températures inférieures à 0 C et donner des résultats comparables, pourvu que la congélation ait lieu aussitôt après la mise en hachis. Lors de la préparation sur grande échelle de muscles réduits en morceaux et désossés provenant de poissons prérigor, il se produira une certaine glycolyse, selon la durée et la température d'entreposage précédant la congélation.

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IN a study of glycolysis and nucleotide changes in prerigor Atlantic cod (*Gadus morhua*) muscle, difficulties were experienced in obtaining samples with similar initial metabolite concentrations even when all were cut from the same fish (Nowlan et al. 1968). Metabolites such as glycogen and high energy phosphate (mainly derived from ATP) are not uniformly distributed throughout the cod fillet (Fraser et al. 1966) and even adjacent transverse slices can differ in glycogen content by as much as 40% (Nowlan et al. 1968). With variations of this magnitude it was necessary to find a way to obtain samples with similar initial concentrations of metabolites if this work was to continue. Mincing has been used to effect a more homogenous distribution of substrates and cofactors in muscle; however, mincing has a stimulatory effect on glycolysis in ox muscle (Newbold and Scopes 1971), on ATP splitting in beef (Hamm and van Hoof 1971; Bendall 1972), and on NAD breakdown in carp (*Cyprinus carpio*) and rainbow trout (*Salmo gairdneri*) (Partmann 1972) if the minces are stored above 0 C.

Because little is known about the effect of mincing on enzymic reactions in marine fish, the rates of glycolysis and loss of high energy phosphate were investigated in prerigor cod muscle slices and in minced muscle during storage unfrozen at 0 C

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and also during freezing and storage at –3 and –6 C to determine the feasibility of using minced samples to replace muscle slices in projected glycolytic studies. The results should also be relevant to understanding possible quality changes occurring in comminuted fish products and in defining proper handling procedures.

Materials and methods — Five feeding cod (designated A,B,C,D, and E) with initial glycogen values between 115 and 800 mg/100 g, representing the range commonly found in unexercised fish at death (Fraser et al. 1966), were selected for this study. They were carefully netted from the laboratory aquarium, immediately stunned by a blow on the head and filleted at once. The fillets were placed in polyethylene bags, submerged in an ice bath, and taken to a refrigerated laboratory at +3 C. A pair of fillets from one fish was used for each experiment; within 10 min of death, transverse slices of white muscle about 1 cm thick were cut from the center of one fillet, and the corresponding part of the other fillet was cut into small pieces and quickly minced in a precooled domestic food chopper (+3 C).

For unfrozen storage at 0 C the muscle slices and mince were put into polyethylene bags and buried in ice. Similar samples for freezing and storage at –3 C were packed into plastic micro weigh-boats 3.6 cm square and 0.8 cm deep to give samples of uniform size and thickness. These were each enclosed in a small polyethylene bag, submerged in an ethylene glycol bath (–10 C) for rapid freezing, then removed when the temperature reached –3 C and transferred to a constant temperature bath at –3 C for storage. Samples for freezing to –6 C were placed in small polyethylene bags, sandwiched between two insulating pieces of fish to insure a gradual and uniform rate of cooling, and frozen for 4 h in circulating air at –17 C, at which time the temperature had reached –6 C. The center layer formed by the mince or slices was about 1 cm thick and the overall pack was equivalent in size and thickness to the commercial 454-g (1 lb) box (see diagram, Nowlan and Dyer 1969). Temperature changes in samples during freezing were monitored by copper-constantan thermocouples and a Honeywell-Brown recording potentiometer.

Acid-soluble metabolites were extracted from 8- to 10-g samples of iced and frozen muscle with ice-cold 0.3 N perchloric acid as described previously (Nowlan

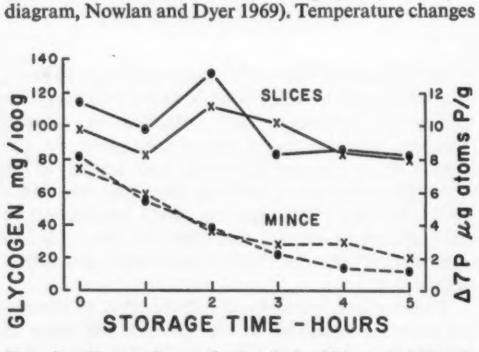

FIG. 1. Comparison of glycolytic (●) and Δ7P (x) catabolism in muscle slices and in minced muscle from fish A at 0 C. Each point is the value for one sample.

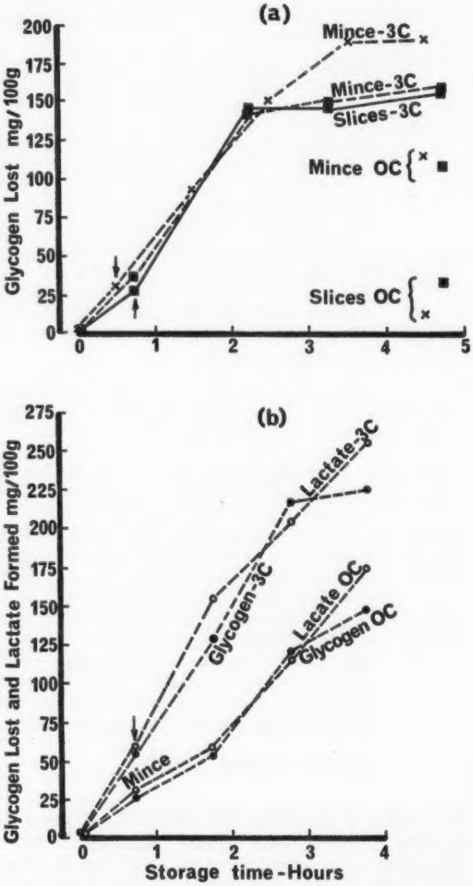

FIG. 2. Glycolysis in prerigor cod muscle mince and slices from the same fish stored unfrozen at 0 C or frozen and held at –3 C. The vertical arrows indicate the point at which the temperature of the freezing samples reached –3 C. a)Results from two experiments with fish B and C. Initial glycogen values in mince and slices, expressed as mg/100 g were: in fish B (■), 165 and 159, respectively; in fish C (x), 250 and 288, respectively. b) Glycogen and lactate changes at 0 C compared with those at –3 C in minced muscle from fish D. Initial glycogen (●) and lactate (○) values were 245 and 95 mg/100 g, respectively.

et al. 1968). The temperature of frozen samples was not allowed to rise prior to extraction. Glycogen was determined as the sum of the acid-extractable fraction and residual glycogen obtained by digesting the tissue residue in 30% KOH (Russell and Bloom 1955). High energy phosphate (Δ7P) which is derived mainly from ATP and creatine phosphate was determined as the increase in inorganic phosphorus after hydrolysis in 1N HCl for 7 min at 100 C (see Nowlan et al. 1968).

Results and discussion — The rates of glycolysis and dephosphorylation of ATP are considerably faster in minced muscle than in unminced samples at 0 C. Figure 1 shows that glycogen and Δ7P were practically depleted in the mince after storage for 5 h in the ice, whereas in the slices from the same fish (A) only small decreases had occurred. It is difficult to compare the actual rates of degradation in the mince and slices because of fluctuations in the curves for glycogen and Δ7P loss in the slices. However, mincing apparently causes at least a threefold increase in glycolysis during storage at 0 C. This agrees with a similar factor of 3 found for ox skeletal muscle (Newbold and Scopes 1971). The increase in the dephosphorylation rate of ATP was somewhat greater (about seven times).

Figure 2 shows losses of glycogen in muscle mince and slices stored unfrozen at 0 C or frozen at –3 C. It was again demonstrated that the loss of glycogen in the unfrozen samples is at least three times greater in the mince than in the slices, using samples from fish B and C with higher initial glycogen levels (160 and 250 mg/100 g, respectively) than in fish A (115 mg/100 g) of Fig. 1. During freezing there was an almost identical increase in glycolytic activity in both mince and slices (Fig. 2a). Figure 2b confirms that the rate of glycolysis (glycogen lost and lactate formed) in minced muscle is greater at –3 C than at 0 C (using samples from fish D). The pattern of Δ7P loss in the iced and frozen muscle slices and mince in Fig. 3 was similar to that of glycogen breakdown in Fig. 2 and the results were comparable among the three fish studied. The activating effect of freezing in the critical temperature zone (–0.8 to –5 C) on these reactions in

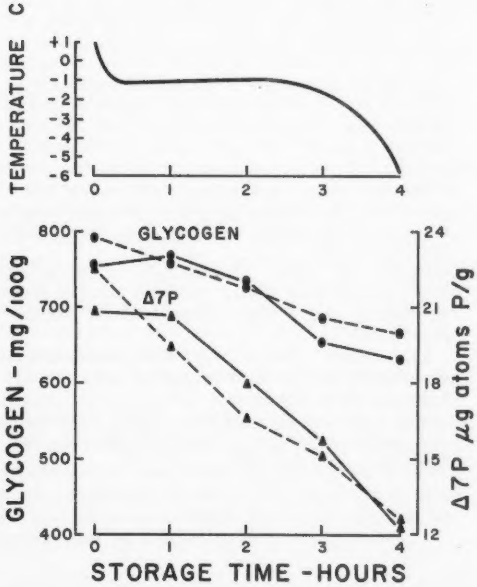

FIG. 3. Loss of Δ7P in prerigor cod muscle slices and minced samples used in Fig. 2. Initial Δ7P values in mince and slices expressed as μg atoms P/g were: (■) 11.7 and 11.5, respectively; (x) 14.2 and 16.5, respectively; (●) 15.7 for the mince in Fig. 3b.

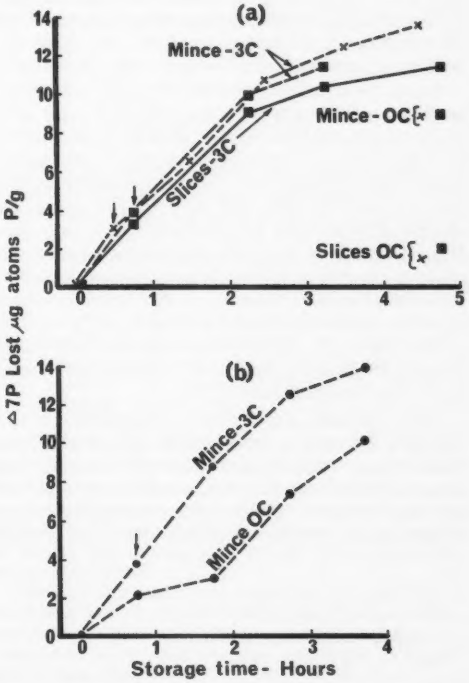

FIG. 4. Changes in the concentration of glycogen and Δ7P in cod muscle slices (solid line) and mince (broken line) during freezing to –6 C. Results from an experiment with a fifth fish, E, are shown.

cod muscle slices has previously been reported (Nowlan and Dyer 1969).

In samples from fish E frozen to -6 C (Fig. 4) the rates of glycolysis and Δ7P loss were similar in the mince and slices, confirming that mincing has no additional stimulatory effect on these reactions when the samples are frozen immediately after mincing. In these experiments mincing appeared to have little effect on the initial levels of glycogen and Δ7P although in Fig. 1 the values for the minced samples were slightly lower.

The stimulatory effect of mincing on rates of glycolysis and ATP breakdown in unfrozen muscle is apparently caused by destruction of the structural integrity of the tissue permitting a more intimate association of enzymes, substrates, and cofactors in the sarcoplasmic matrix. Activation of ATP splitting in minced chicken muscle has been attributed to disruption of the sarcoplasmic reticulum and liberation of calcium ions which activate the ATP hydrolase (see Partmann 1969).

The acceleration of reaction rates in both mince and slices during slow freezing within the critical zone is probably caused by increasing concentrations of substrates and electrolytes (see Love 1966 as well as injury to membranes and other structures.

From these results with unexercised cod representing a wide range of initial glycogen and Δ7P levels we conclude that minced muscle can be used to provide samples of homogenous experimental material for glycolytic studies at subzero temperatures and that the resulting data will be typical of unminced muscle. However, in experiments where unfrozen minced muscle is used, the subsequent stimulatory effect of mincing on glycolytic activity must be taken into consideration. Because of the large variation in initial metabolite levels, glycogen, Δ7P, etc., in different fish, it is difficult to average the results and so only characteristic results of a number of experiments are reported here.

These findings have direct application to the deboning process in comminuted fish production. With prerigor muscle, glycolysis will occur during passage through the deboning machine, the reaction rate depending on the time delay and temperature of processing. Further glycolysis will occur during subsequent freezing of the product influenced by the freezing rate (time in the thermal arrest zone between -0.8 and -5 C, Nowlan and Dyer 1969), but the present results indicate that rates in the deboned frozen minced muscle will be no greater than in intact muscle.

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Preparation and Use of a Modified Protein Binder in the Fabrication of Feed for Post-Larval Aquacultural Animals

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GRONINGER, H. S. JR., AND R. MILLER. 1974. Preparation and use of a modified protein binder in the fabrication of feed for post-larval aquacultural animals. J. Fish. Res. Board Can. 31: 477–479.

The laboratory preparation of a modified protein (acylated) binder is described. The important feature of this binder is that it enables a rapid and simply effected conversion of a liquid feed to a solid feed. A laboratory method is given for the fabrication of aquacultural feed using this modified binder. A typical feed that has been prepared with this binder has an elastic texture, resists fragmentation in water, and is readily acceptable to test animals.

GRONINGER, H. S. JR., AND R. MILLER. 1974. Preparation and use of a modified protein binder in the fabrication of feed for post-larval aquacultural animals. J. Fish. Res. Board Can. 31: 477–479.

Nous décrivons la préparation en laboratoire d'un liant protéique modifié (acylé). Une caractéristique importante de ce liant est de permettre une conversion rapide et simple d'une nourriture liquide en une nourriture solide. Nous décrivons une méthode de laboratoire pour la fabrication, à l'aide de ce liant, de nourriture utilisée en aquiculture. Une nourriture typique préparée avec ce liant a une texture élastique, est résistante à la fragmentation au contact de l'eau et est facilement acceptée par les animaux témoins.

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WHILE studying the modification of fish proteins (Groninger 1973), it was observed that the properties of this modified protein could be utilized in a binder in aquacultural feeds. This protein, that has been modified by acylation of a portion of its free amino groups, could be converted from a liquid to a gel-like solid by adjusting the *p*H from neutrality to about 4.5 or lower. This property was sufficiently strong that a mixture of feed material and the modified protein could be converted to a continuous elastic solid that maintained its form during handling and immersion in water.

This note describes work done to establish the potential usefulness of this modified protein as a binder for specialized aquacultural feeds. Specifically, the binder can be utilized in the fabrication of a number of types of rations that contain a variety of feed ingredients. Some of the properties of these rations will be determined and some selective test feeding will be carried out.

Methods — Preparation of modified protein binder — In general, the modified protein binder is prepared by acylating whole muscle or preferably myofibrillar protein from poultry, meat, or fish. The details of preparing myofibrillar protein from fish are: Flesh is removed from the fish carcass by hand filleting or by use of a flesh-separating machine. The flesh is comminuted by a single pass through a food grinder equipped with a plate having approximately 0.15-cm orifices. The soluble muscle protein is removed from the myofibrillar protein by suspending the comminuted muscle (three times) in 5–10 m^3 of 0 C, 0.5% NaCl solution. The insoluble protein is collected in a cheesecloth filter. This protein is suspended in 2–3 m^3 of 0 C, 0.5% NaCl solution in a high-speed blender for 15–20 s, and additional soluble protein is removed by centrifugation at 13,000 × *g*. Three parts of the sedimented material, which contains mainly myofibrils, is suspended in seven parts of 3.5% NaCl solution at 0 C and blended in a high-speed blender for about 1 min. This will give a protein dispersion that contains about 4% protein, dry weight. This is about the maximum concentration of protein that can be easily stirred in the subsequent acylation reaction.

The acylation reaction is carried out at 0.0–25.0 C, preferably at 0 C, and at a *p*H between 8.0 and 10.0, preferably at 8.5, by reacting a protein dispersion or suspension with solid succinic anhydride or liquid acetic anhydride.

The following types of protein preparations can be acylated for use as binders: dispersions of myofibrillar protein, comminuted whole muscle that is dispersed in 3.5% NaCl solution, and muscle myofibrils or comminuted whole muscle that are suspended in 0.5%

NaCl solution. Acylated binders prepared from dispersions of myofibrillar protein have properties superior to those of dispersed whole muscle protein. These in turn have better properties than binders prepared by acylation of proteins suspended in 0.5% NaCl solution.

In practice, the acylation reaction is carried out by adding incremental amounts of anhydride to the protein during the first 25–75 min of the 1–2 h reaction period. Approximately $\frac{1}{5}$–$\frac{1}{10}$ of the total amount of anhydride is added each time and the dispersion is stirred at a rate sufficient to distribute the anhydride throughout. The *p*H is maintained by the addition of 2 *N* NaOH. After the last addition of anhydride, the amount of NaOH that is added to the reaction mixture is decreased so that the final *p*H of the reaction is about 7.0. Ratios of 1:5–1:10 of anhydride to protein are normally used (protein was determined by the method of Lowry et al. 1951). To have binding properties, the acylated protein should have not less than 30% of its total lysine reacted with the anhydride, and to have good binding properties, about 50–70% of the lysine groups should be reacted with anhydride (the extent of the reaction with lysine was estimated by the method of Kakade and Liener 1969).

The binding characteristics of an acylated protein preparation can be evaluated at intervals during the acylation reaction. This evaluation is carried out by expressing a 10-ml volume of the protein dispersion into a magnetically stirred bath containing a 5% solution of citric or acetic acid. For this purpose, a syringe with an orifice of approximately 3.1-mm diameter is used. To have acceptable binding characteristics, the extruded fiber that is formed in this test should have sufficient tensile strength so that approximately a 30-cm length of fiber can be held and lifted by one end without breaking, and the test acid bath should be relatively free of particulate protein.

Fabrication of experimental feed — The acylated protein, as a viscous dispersion that contains about 4% protein, is thoroughly blended with the other feed constituents and the mixture is extruded or prilled to obtain the desired final form of the feed.

To prepare a typical batch of feed, 200 g of acylated protein dispersion is blended with approximately 32 g, dry weight, of any finely ground feed material. For this purpose, a high shear such as a Lourdes[1] Volu-Mix homogenizer or a multi-speed kitchen-type blender is used. High-shear blending appears to give the feed mixture a more desirable texture. Total blending time is 3–4 min at low-to-medium speed and up to 2 min or more at high speed. To obtain a feed that has good stability in water and has a somewhat elastic texture, the mixture should contain approximately 15% of the acylated protein on a dry weight basis.

If the feed is being prepared for a bottom-feeding animal, the air entrapped in the mixture during the blending step is removed by evacuation of the blended mixture. This is best accomplished immediately after the blending step, while the viscosity of the mixture is still low. A typical procedure for removing entrapped air is to transfer the blended mixture into a 1-liter filtering flask, warm the flask to 60–80 C, evacuate the flask using a filter pump, and agitate the contents of the flask to remove the air bubbles.

To put the deaerated mixture into the desired final form, the mixture is either prilled or extruded into a dilute organic acid.

A prilled product is prepared by pouring the deaerated mixture, discontinuously, into a 600-ml beaker containing 400 ml of a 5% solution of acetic or citric acid. The shape and size of the prilled product is largely determined by the consistency of the mixture and the height from which the liquid mixture is poured into the acid bath. The prilled product is held in the acid for up to 30 min; then it is washed briefly, two times, in approximately 200 ml of water and allowed to drain before it is refrigerated or frozen.

An extruded feed or fiber is prepared by expressing the deaerated mixture from a syringe that has an orifice of approximately 3.1-mm diameter. Care is exercised in filling the syringe to avoid the entrapment of air. The bath used for extrusion is a 600-ml beaker containing 400 ml of a 5% solution of citric or acetic acid maintained at ambient or lower temperature. The bath is stirred magnetically at a rate just sufficient to draw the extruded product from the tip of the syringe. The extruded feed is formed by holding the tip of the syringe just under the surface of the acid and expelling the mixture into the bath. Once an extruded fiber containing approximately 12–36 ml of mixture has been formed, the bath is removed from the stirrer and the extruded feed is held in the acid for approximately 5 min; then it is washed briefly, two times, in approximately 200 ml of water. The feed is drained, blotted dry, and then refrigerated or frozen.

TABLE 1. Proximate analysis of a feed prepared with succinylated myofibrillar protein and Oregon Pellet No. 2.[a]

Feed constituent	% Wet basis	% Dry basis
Moisture[b]	79.1	–
Protein[b]	12.7	61.0
Fat[c]	3.5	16.5
Ash[b]	0.8	3.8
Carbohydrate (by difference)	3.9	18.7

[a] Feed was prepared with 44 g Oregon Pellet No. 2 and 200 g of succinylated fish myofibrillar protein dispersion that contained approximately 4% protein.

[b] Moisture, protein, and ash were determined by procedures of the Association of Official Agricultural Chemists (1970).

[c] Fat was determined by extraction of the dried feed for 12 h using a solvent mixture of methanol:chloroform (2:1, v/v).

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[1] Use of trade names does not imply endorsement of commercial products.

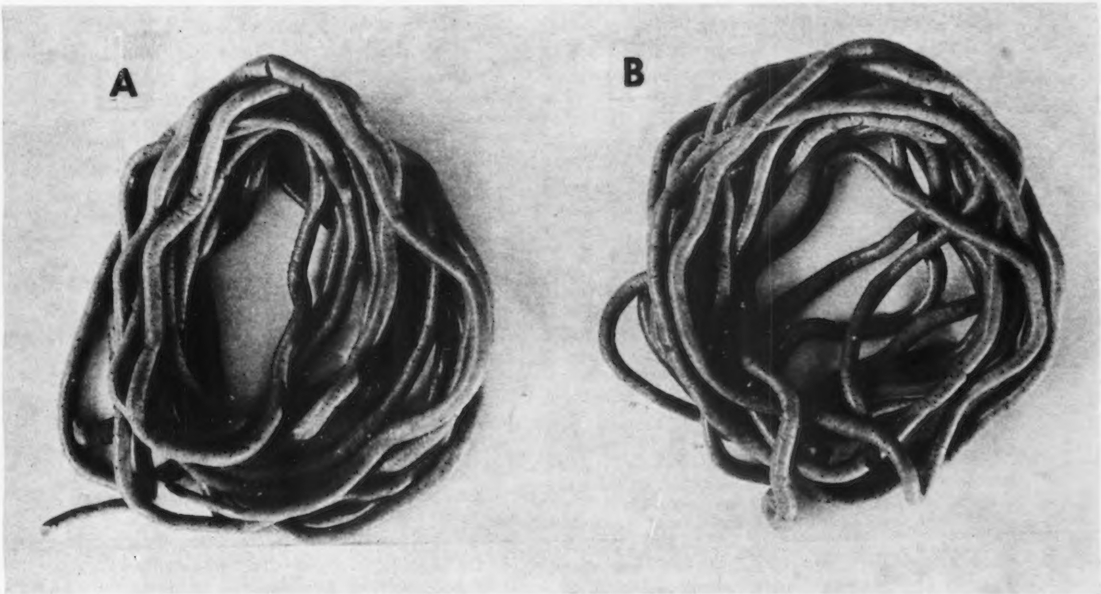

FIG. 1. Extruded feed prepared with 44 g Oregon Pellet No. 2 and 200 g succinylated fish myofibrillar protein dispersion that contained approximately 4% protein. A. Control B. After immersion in sea water for 24 h.

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Results and discussion — The laboratory methods given for the preparation of the binder and its fabrication into a feed are methods that have been demonstrated as useful for preparing certain specialized feeds. If larger than laboratory quantities of binder or feed are required, both methods have the potential for scale-up to fulfill these requirements. Due to their high moisture content, these feeds which have been prepared with modified protein binder would most likely be prepared at the feeding site.

The important feature of the binder is that it enables the rapid and simply effected conversion of a liquid feed into a solid feed of a variety of shapes and sizes. It is this unique feature that represents an apparent advantage over some of the other binders that have been used (Meyers et al. 1972; Sick et al. 1972; and Thain and Urch 1972).

The binder gives a feed that has considerable mechanical strength, a gel-like and somewhat elastic texture, a high moisture content, and a surface that is moist but not sticky. This feed can be frozen and thawed; however, this does result in the loss of some moisture and in a slightly firmer texture. The feed resists disintegration when it is manipulated by the animal prior to eating. It does not fragment when held in sea water for a period of 24 h (Fig. 1); however, there is some leaching of soluble constituents from the feed during immersion in water. A typical analysis of a feed prepared using the modified protein binder and Oregon Pellet No. 2 is given in Table 1.

It has been demonstrated that a wide range of feed materials can be bound using the modified protein binder. In our laboratory, Oregon Pellet, Purina shrimp feed, dried pelagic red crab, and comminuted mussel have been used successfully. Also, preliminary tests have shown that two therapeutic agents, polyvinylpyrrolidone iodine and a vaccine against kidney disease in fish, can be bound into a feed using the modified protein binder.

Feed samples have been prepared for the lobster (*Homarus americanus*), the spot shrimp (*Pandalus platyceros*), and the rainbow trout (*Salmo gairdneri*). For each of these species, preliminary feeding trials have indicated that feed prepared with the modified protein binder is readily acceptable to the animals and that the animals continue to consume the feed. Longer-term feeding studies with the spot shrimp showed that the feed prepared with this binder gave reasonably good growth and good viability when compared to an appropriate control.

Estimates of the cost of the modified protein binder are difficult to make because it is an experimental material that is not available commercially. However, it is not unreasonable to suggest that this binder could be prepared at a cost similar to the cost of the more expensive binders that are presently used to prepare rations for experimental aquacultural animals.

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Toxicity of Various Formulations of 2,4-D to Salmonids in Southeast Alaska[1]

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Meehan, W. R., L. A. Norris, and H. S. Sears. 1974. Toxicity of various formulations of 2,4-D to salmonids in southeast Alaska. J. Fish. Res. Board Can. 31: 480–485.

To determine acute toxicity to juvenile (1) pink, chum, coho, and sockeye salmon, (2) Dolly Varden char, and (3) rainbow trout, 2,4-D acid, butyl and isooctyl esters were tested in southeast Alaska. A comparable test was made in Oregon using coho salmon fingerlings. The mean percent mortality after 96 h and the highest concentration of herbicide that did not produce any mortality were determined for each formulation tested.

At less than 50 ppm 2,4-D acid produced no mortality except in pink salmon fry. The butyl ester was most toxic causing nearly complete mortality in all species at concentrations > 1.0 ppm and the isooctyl ester least toxic of the ester formulations. Alaskan and Oregon coho fingerlings were similar in their responses to 2,4-D acid, butyl and isooctyl esters. The toxicities of three different formulations of isooctyl ester, a PGBE ester, and butyl ester to Alaskan coho fingerlings were also determined. There were few or no differences in toxicity among isooctyl ester formulations. The butyl and PGBE esters were similar in toxicity.

Meehan, W. R., L. A. Norris, and H. S. Sears. 1974. Toxicity of various formulations of 2,4-D to salmonids in southeast Alaska. J. Fish. Res. Board Can. 31: 480–485.

Nous avons fait des essais avec le 2,4-D acide et les esters de butyle et d'isooctyle dans le sud-est de l'Alaska en vue de déterminer leur toxicité aiguë sur les jeunes (1) des saumons rose, keta, coho et nerka, (2) de la Dolly Varden et (3) de la truite arc-en-ciel. Un essai semblable a été effectué dans l'Orégon sur des alevins de la grosseur du doigt de saumon coho. Le pourcentage moyen de mortalité après 96 h et la plus forte concentration d'herbicide qui n'entraîne pas de mortalités ont été déterminés pour chaque formulation essayée.

Le 2,4-D acide à moins de 50 ppm ne produit pas de mortalités sauf chez les alevins de saumon rose. L'ester de butyle est le plus toxique, causant des mortalités presque complètes chez toutes les espèces à des teneurs de > 0.1 ppm. L'ester d'isooctyle est la moins toxique de toutes les formulations essayée. Les alevins de la grosseur du doigt des saumons coho de l'Alaska et de l'Orégon réagissent de la même façon au 2,4-D acide et aux esters de butyle et d'isooctyle. Nous avons également déterminé la toxicité de trois formulations différentes d'ester d'isooctyle, d'un ester de PGBE et de l'ester de butyle sur des alevins de saumon coho de l'Alaska. Les différentes formulations d'ester d'isooctyle ne montrent que peu ou pas de différences. Les esters de butyle et de PGBE ont une toxicité semblable.

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[1] This publication reports research involving pesticides. It does not contain recommendations for their use nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended.

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MOST Alaskan fish populations have never been exposed to herbicides and they occupy waters with unique chemical and physical properties. There have been no previous tests of the toxicity of formulations of 2,4-D to Alaskan races of fish in their native waters and research has been inadequate to justify extrapolation of toxicity data from studies conducted in other parts of the United States to the Alaskan environment. Because the U.S. Forest Service is using 2,4-D for vegetation control in southeast Alaska, the present study was undertaken to test the toxicity of the chemical to salmonid fishes indigenous to the forested streams in this region (Sears and Meehan 1971).

In determinations of toxicity of pesticides to fish the phenoxy herbicides (2,4-D, 2,4,5-T, and 2,4,5-TP) have received considerable attention (Hughes and Davis 1963; Rawls 1965; Bond et al. 1960). These herbicides are available as esters, amines, or inorganic salts in a wide variety of commercial formulations. There are marked differences in toxicity to fish among formulations (Hughes and Davis 1963) and selection of formulations for use in the field should take into account the toxicity of specific formulations, as well as the toxicity of a general class of herbicide.

Material and methods — Chemicals — The herbicides used in these tests were: (1) purified 2,4-D acid; (2) Monsanto Crop Guard Weed Killer (*n*-butyl, isobutyl ester of 2,4-D[2]); (3) Monsanto 2,4-D Low Vol. Ester Weed Killer (isooctyl ester of 2,4-D); (4) Dow propylene glycol butyl ether (PGBE) ester of 2,4-D; (5) Diamond isooctyl ester of 2,4-D; and (6) Rhodia isooctyl ester of 2,4-D. Monsanto technical grade 2,4-D acid was recrystalized three times from benzene to obtain the purified 2,4-D acid. All other herbicides were used as received from the manufacturer and, we assume, are representative of these materials as available for field use.

Test animals — Resident fish of economically important species were chosen for testing. The species used in these experiments were: coho salmon, *Oncorhynchus kisutch* — fry and fingerlings; chum salmon, *O. keta* — fry; pink salmon, *O. gorbuscha* — fry; sockeye salmon, *O. nerka* — smolts; Dolly Varden, *Salvelinus malma* — several age-classes; and rainbow trout, *Salmo gairdneri* — several age-classes.

The fish were livetrapped in streams in the Juneau–Auke Bay vicinity. Rainbow trout were obtained from Baranof Island, about 120 miles south of Juneau. The fish were transported immediately after capture to the laboratory at Auke Bay.

All test animals were acclimated for 3–10 days in tanks. Fish that displayed abnormal behavior were not used as test animals.

Water chemistry — Water used during the course of the study was unfiltered and untreated, taken from a depth of 12.19 m (40 ft) in Auke Lake near the laboratory. Several indices of water chemistry were measured during March and September, 1970 and June 1971, using methods of Rainwater and Thatcher (1960). Samples of the water were also analyzed for background residues of chlorinated hydrocarbon insecticides and s-triazine and phenoxy- and picloram-type herbicides (Burchfield and Johnson 1965; Mattson et al. 1965; Anon. 1968).

Herbicide loss during testing — The influence of aeration and of presence of fish on persistence of 2,4-D during a 96-h exposure period was determined in bioassay jars each of which contained either 1 ppm acid equivalent 2,4-D acid, or butyl, or isooctyl ester The experiment was run in triplicate.

Water was drawn from the bioassay jars 24, 48, 72, and 96 h after introduction of herbicide. Quart samples were placed in glass jars containing 15 g of sodium hydroxide. Samples were then acidified to *p*H 1.0 with hydrochloric acid and extracted three times with 100-ml portions of benzene. The benzene extract was evaporated to dryness and methylated with diazomethane. Samples were diluted as needed with benzene and were assayed with a Microtech 2000 gas chromatograph. The 4-mm-ID × 1.2-m glass column was packed with 6% OV-1 coated, 60- to 80-mesh, Gas Chrom Q. The column was operated at 150 C with a flow of N_2 carrier gas of 90 ml/min at 40 psi. The herbicide was detected with the chlorine specific Infotronics T-300 microcoulometric detector. The precision of the analytical method is ±5%.

Bioassay methods — Modified bioassay methods of Doudoroff et al. (1951) and the Pesticides Regulation Division (1969) were used as guidelines in setting up the experiment. Wide-mouthed, 18.9-liter (5-gal) glass jars were used as containers and were arranged in rows of five in constant temperature water baths. All experiments were conducted in triplicate at 10 ± 1 C.

Each jar was fitted with a paraffin-impregnated plywood lid. We provided uniform aeration to all jars from an oilless compressor. Filtered air was pumped to each jar through glass tubing that was fitted with a disposable bubbler.

Fifteen liters of water were measured into each jar and allowed to reach 10 C before the chemicals were added. Aeration of the water was started at this time. Stock solutions of equal concentration of each herbicide formulation were prepared immediately before the test media were inoculated. Water was withdrawn from each jar to equal the volume of stock solution to be added. Because pure 2,4-D acid has a low solubility in water, dilutions were prepared by weighing the required amount of pure acid into each jar and adding

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[2] Mixtures of *n*-butyl, isobutyl esters of 2,4-D are referred to as "butyl" ester. Use of trade names does not imply endorsement by the U.S. Department of Agriculture.

15 liters of water. Considerable stirring was required to dissolve the acid crystals.

Test solutions consisted of concentrations of 1, 5, 10, and 50 ppm of each chemical. Ten fish were placed in each jar. The mass of fish per jar was between 1.6 and 2.8 g of fish per liter. Fish were introduced into the test medium within 45 min after the introduction of the chemical. Observations of fish mortality were recorded 24, 48, 72, and 96 h after introduction of the fish. A fish was considered dead when all movements had ceased and no further responses could be obtained by touching or moving the fish. Fish that died during the tests were removed immediately.

After completion of each test, the jars and lids were rinsed with isopropyl alcohol, washed and rinsed twice in hot water and strong detergent, rinsed with acetone followed by hot water, and then rinsed twice with untreated lake water. The jars were then refilled with 15 liters of water and replaced in the water bath.

To evaluate applicability of bioassay work to widely different geographical locations, toxicity tests were done in Corvallis, Ore. using native coho salmon fingerlings, but using chemicals from the same containers used in Alaska. All test conditions were the same as those for the Alaskan tests.

Results and discussion — Chemical characteristics of the test water were generally similar throughout the study. Hardness as calcium and magnesium ranged from 10.0 to 33.64 ppm during the study period; such differences have no significant effect on toxicity of various organic herbicides, including 2,4-D (Ingles and Davis 1970).

Assay of test water for background pesticide residues showed no detectable quantities of s-triazine, phenoxy- or picloram-type herbicides, or the insecticides endrin, aldrin, dieldrin, hep-

TABLE 1. Mean percent mortality of fish after 96-h exposure to 2,4-D acid, butyl or isooctyl esters in static water toxicity tests.

Species	2,4-D	ppm 2,4-D a.e.[a]				N.E.[b] level (*ppm*)
		1	5	10	50	
Pink fry	Pure acid	10.0(0–20)[c]	13.3(10–20)	43.3(0–90)	100.0(0)	<1
	Butyl ester	100.0(0)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	40.0(0–70)	80.0(50–100)	100.0(0)	100.0(0)	<1
Chum fry	Pure acid	0.0(0)	0.0(0)	0.0(0)	66.7(0–100)	10
	Butyl ester	100.0(0)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	66.7(30–90)	60.0(20–100)	100.0(0)	1
Coho fry	Pure acid	0.0(0)	0.0(0)	0.0(0)	80.0(40–100)	10
	Butyl ester	100.0(0)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	6.67(0–10)	13.3(0–30)	100.0(0)	1
Sockeye smolts	Pure acid	0.0(0)	0.0(0)	0.0(0)	6.7(0–20)	10
	Butyl ester	70.0(10–100)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	6.7(0–20)	33.3(20–50)	63.3(20–100)	100.0(0)	<1
Alaska coho fingerlings	Pure acid	0.0(0)	0.0(0)	0.0(0)	0.0(0)	50
	Butyl ester	6.7(0–20)	93.3(80–100)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	0.0(0)	3.3(0–10)	40.0(10–60)	5
Dolly Varden fingerlings	Pure acid	0.0(0)	0.0(0)	0.0(0)	0.0(0)	50
	Butyl ester	16.7(10–30)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	0.0(0)	0.0(0)	66.7(30–100)	10
Rainbow fingerlings	Pure acid	0.0(0)	0.0(0)	0.0(0)	0.0(0)	50
	Butyl ester	53.3(30–70)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	0.0(0)	0.0(0)	63.3(40–80)	10
Oregon coho fingerlings	Pure acid	0.0(0)	0.0(0)	0.0(0)	73.3(50–100)	10
	Butyl ester	26.7(0–50)	100.0(0)	100.0(0)	100.0(0)	<1
	Isooctyl ester	0.0(0)	0.0(0)	0.0(0)	20.0(10–30)	10

[a] a.e. — acid equivalent.

[b] N.E. — the no acute effect level is the highest concentration tested that produced no mortality in test fish.

[c] (0–20) — range in percent mortality in three replications.

tachlor, heptachlor epoxide, DDT[3], DDD, or DDE. The analytical method used is capable of quantitatively detecting 0.001 ppm of these pesticides. Therefore we feel that responses of our bioassay fish were only to the herbicides being tested.

Doudoroff et al. (1951) preferred not to aerate test solutions unless absolutely necessary, because they believed that chemical loss might result. In our tests, 45–50 mm coho (age 0+) died within 96 h when held in bioassay jars at 10 C without aeration. With aeration, 10 fish up to 150 mm in length could be held for 96 h in 15 liters of water with no apparent ill effects.

Measurements of persistence of 2,4-D pure acid, butyl ester, and isooctyl ester as influenced by aeration and fish showed no statistically significant difference among concentrations of herbicide subjected to different treatments after 96 h of exposure.[4] Some small "loss" of chemical occurred in the first 24-h period in all treatments, but may have been more apparent than real because these residues are as measured in the water and do not take into account herbicide that is reversibly adsorbed on various surfaces in the system. Some investigators suggest a maximum of 2 g of fish per liter of test solution to prevent depletion of test chemicals due to uptake or metabolism by the fish (Doudoroff et al. 1951). Because neither aeration nor presence of fish significantly decreased the concentration of any of the three herbicides tested, we feel the use of aerated solutions and up to 2.8 g of fish per liter of test solution was justified.

Because of the wide spread of concentrations tested in this study, much of the data shows either complete mortality or survival after 96- h exposure. These results precluded calculation of TL_m in the usual manner. Similarly, lack of variance where complete mortality or survival occurred also prevented statistical comparisons of percent mortality among treatments. However, the essential elements of our results are clear. Mean percent mortality (three replications) of test fish after 96-h exposure to various concentrations of chemical are shown in Table 1. There were some striking differences in toxicity among the chemicals tested. The butyl ester is much more toxic than the isooctyl ester or pure 2,4-D acid. The no-effect level in Table 1 is the highest concentration tested that caused no mortality in 96 h in any of the three replications. The no-effect level for butyl ester is considerably less than 1 ppm as relatively high mortality occurred at that level for nearly every species tested. The no-effect level for isooctyl ester ranged from 1 to 10 ppm for all fish except pink salmon fry which had 40% mortality at 1 ppm and sockeye smolts with 7% mortality at 1 ppm.

The 2,4-D is the herbicidally active component of each chemical tested; thus the chemical form of the herbicide and constituents of formulation have far

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[3] Trace amounts of DDT were observed, but the level was less than 0.001 ppm.

[4] Data were analyzed by multivariate analysis of variance.

TABLE 2. Mean percent mortality of Alaskan coho fingerlings after 96-h exposure to 2,4-D acid, butyl, isooctyl, or PGBE[a] esters in static water toxicity tests.

2,4-D	ppm 2,4-D a.e.[b]				N.E.[c] level (*ppm*)
	1	5	10	50	
Pure acid	0.0(0)[d]	0.0(0)	0.0(0)	0.0(0)	50
Isooctyl ester (Monsanto)	3.3(0–10)[d]	0.0(0)	3.3(0–10)	83.3(60–100)	<1
Isooctyl ester (Diamond)	3.3(0–10)	0.0(0)	3.3(0–10)	96.7(90–100)	<1
Isooctyl ester (Rhodia)	0.0(0)	3.3(0–10)	16.7(10–30)	90.0(70–100)	1
PGBE ester (Dow)	26.7(0–80)	100.0(0)	100.0(0)	100.0(0)	<1
Butyl ester (Monsanto)	6.7(0–20)	93.3(80–100)	100.0(0)	100.0(0)	<1

[a] PGBE — propylene glycol butyl ether.

[b] a.e. — acid equivalent.

[c] N.E. — the no acute effect level is the highest concentration tested that produced no mortality in test fish.

[d] (0); (0–10) — range in percent mortality in three replications.

more impact on fish than the 2,4-D alone. Toxicity data for specific formulations of herbicide should be consulted when selecting formulations for use in the field.

Various species of fish of similar size reacted generally the same to similar concentrations and formulations of 2,4-D. If pink, chum, and coho salmon fry are considered as one group and the older (and larger) rainbow, Dolly Varden, and coho fingerlings considered as another group, then the fry appear to be more sensitive to all three formulations. Sockeye smolts were intermediate between these two groups in sensitivity. Post and Schroeder (1971) found that various pesticides were more toxic to fish of lower body weight.

The tests on Oregon coho fingerlings showed that these fish behaved generally the same as the Alaskan cohos of similar size and age (Table 1).

Our test results for coho and rainbow trout are similar to results reported by other investigators (Bond et al. 1960; Kibby 1966) but this is the first report for other species and races of Alaskan fish. Alaskan land managers have felt that fish toxicity tests conducted in other parts of the United States might not apply to Alaskan fish in their natural environments. The similarities between our results and the results of others with coho salmon and rainbow trout and the direct comparison we made of the response of Oregon and Alaskan coho fingerlings make it clear that land managers can use a wide variety of fish toxicity test results in selecting chemicals for use in the coastal Alaskan environment.

Tests with aquatic insects yielded very erratic results and we found fish more reliable as indicators of toxicity of 2,4-D to stream organisms.

The relatively low toxicity of the isooctyl ester in comparison with the butyl ester prompted us to investigate the toxicity of several low volatile ester formulations. Isooctyl ester formulations from three manufacturers and the PGBE ester from one manufacturer were included to test for differences in toxicity among these products (Table 2).

There were no striking differences in toxicity among formulations of isooctyl ester, but the PGBE ester approached the butyl ester in toxicity (Table 2). Hughes and Davis (1963) found differences in toxicity of a given 2,4-D ester formulated by different manufacturers. They used warmwater fish and a narrower range of herbicide concentrations, and the differences in toxicity noted were not large in most instances. Our data do not provide a basis for choosing among formulations of isooctyl ester for field use, but clearly the butyl and PGBE ester formulations pose a more significant hazard to fish than the isooctyl esters if direct application of the chemical to surface water occurs.

It is doubtful that there are large degrees of difference in the effectiveness of various phenoxy herbicide esters in terms of brush control on forest lands (Hull 1967). Therefore, selection of specific phenoxy herbicide ester formulations should be made on the basis of the possible impact on aquatic organisms when the probability exists that the chemical will enter the water (Juntunen and Norris 1972).

Our study determined only relative "acute" toxicity among formulations of 2,4-D, and gave no information on "chronic" effects, such as reduced fecundity and growth potential, changes in body chemistry or genetic makeup, or alteration of behavioral characteristics.

The sex, size, life history (e.g. hatchery-reared vs. wild), condition, and acclimation of fish subjected to bioassay tests and various chemical properties of test water are factors that should receive more thorough consideration in testing of this type (Mount 1966; Hughes and Davis 1963; Sprague 1970; Cope 1971). Sprague (1969, 1970, 1971) reviewed in depth the methods for measuring toxicity of chemicals to fish. In addition, more research needs to be conducted on the various substances (e.g. surfactants and oil carriers) that are used operationally with chemicals like 2,4-D. In some cases, more than one chemical may be used in one area, and the synergistic effects of combinations of such chemicals deserve investigation.

The butyl ester and the PGBE ester of 2,4-D were considerably more toxic to fish than was the isooctyl ester in our tests. In general, the least toxic material that is effective should be recommended for use where there is any possibility that the chemical may enter the aquatic environment. No major differences were found in sensitivity among species of salmonids of comparable age or size exposed to a given chemical. Hence, bioassay work may be confined to the most readily available species. Results of the Oregon and Alaska tests were similar; therefore when circumstances make it impossible to undertake separate investigations, reasonable inferences may be made from other pesticide studies to the Alaskan freshwater environment.

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Chronic Toxicity of NTA (Nitrilotriacetate) and Metal-NTA Complexes to *Daphnia magna*

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Biesinger, K. E., R. W. Andrew, and J. W. Arthur. 1974. Chronic toxicity of NTA (nitrilotriacetate) and metal-NTA complexes to *Daphnia magna*. J. Fish. Res. Board Can. 31: 486–490.

The chronic toxicity of NTA (nitrilotriacetate) tested as the trisodium salt, to *Daphnia magna* in eight natural waters varied tenfold. A strong negative correlation ($r = 0.95$) between water hardness and NTA toxicity was found; NTA was most toxic in water with a total hardness of 22 mg/liter and was least toxic in water with a total hardness of 438 mg/liter. The 21-day LC50 in all cases closely approximated the calculated molar equivalence point of total hardness and NTA. Bioassay experiments of NTA, with copper and zinc added, confirmed earlier reports that chelates of copper and zinc with NTA are relatively nontoxic. The solutions were toxic only when the metals or NTA were present in excess. The toxicity of iron (III) was not appreciably changed by NTA chelation.

Biesinger, K. E., R. W. Andrew, and J. W. Arthur. 1974. Chronic toxicity of NTA (nitrilotriacetate) and metal-NTA complexes to *Daphnia magna*. J. Fish. Res. Board Can. 31: 486–490.

La toxicité chronique du NTA (nitrilotriacétate) essayé sous forme de sel de trisodium sur *Daphnia magna* dans huit eaux naturelles varie de dix ordres de grandeur. Nous avons trouvé une forte corrélation négative ($r = 0.95$) entre la dureté de l'eau et la toxicité du NTA; NTA a une toxicité maximale dans l'eau de dureté totale de 22 mg/litre et une toxicité minimale dans l'eau de dureté totale de 438 mg/litre. Dans tous les cas, la LC50 après 21 jours se rapproche étroitement du point d'équivalence molaire calculée de la dureté totale et du NTA. Des essais biologiques avec NTA auquel on a ajouté du cuivre et du zinc confirment des rapports antérieurs à l'effet que les chélates du cuivre et du zinc avec NTA sont relativement non toxiques. Les solutions ne sont toxiques que lorsqu'il y a excès de métaux ou de NTA. La toxicité du fer (III) n'est pas affecté de façon appréciable par la chélation avec le NTA.

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Several investigators have determined the toxic effects of NTA (as the sodium salts) to aquatic animals. Acute lethal concentrations of NTA were found by Weaver (1970), Arthur et al. (1974), Flannagan (1971), and Sturm and Payne (1973). Flannagan (1971) tested the 96-h median tolerance limits of 17 species of macroinvertebrates and two species of amphibians and concluded that NTA was relatively nontoxic. Arthur et al. (1974) tested the effects of Na_3NTA through complete life-cycle exposures in flowing systems and found that the no-effect level was about 18 mg/liter for an amphipod species and >50 mg/liter for the fathead minnow (*Pimephales promelas*).

Few papers have been published on the toxicological interactions of NTA with substances commonly found in natural waters, although Sprague (1968) reported that NTA chelated copper and zinc and greatly reduced the toxicity of these metals. He recommended NTA as a possible antipollutant for incidents of accidental spills of copper and zinc. Sturm and Payne (1973) reported that acute toxicity varied with water hardness. Flannagan (1971) discussed the effects of water hardness, chelation, and *p*H and concluded that in acute tests mortality in most, if not all, cases resulted from increases in *p*H. The findings of Arthur et al. (1974) also indicated thta acute toxicity resulted from increased *p*H.

We investigated the toxicity of the trisodium salt of nitrilotriacetate (Na_3NTA) to *Daphnia magna*. Certain chemical interactions with metallic cations found in natural waters were also studied for assessing the possible impact of this compound on the aquatic environment.

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Materials and methods — Bioassay experiments with laboratory-reared *Daphnia magna* were conducted for 3 wk (more than a complete generation cycle which is 8–10 days at 18 C, the test temperature) in 250-ml beakers containing 200 ml of test water. The chronic test methods of Biesinger and Christensen (1972) were used. Five daphnids less than 24 h old were placed in each of four beakers with a total of 20 animals for each experimental condition and control: experiments were often replicated. The results for survival are given as 3-wk 50% lethal concentrations (LC50) as recommended by Sprague (1969). Statistical evaluations were made by using the method of Litchfield and Wilcoxon (1949) for establishing approximations of the 95% confidence limits where three or more partial-effect concentrations were tested. Effects on reproduction were analyzed in a similar manner, excluding confidence limits, but are reported in terms of reproductive impairment by percentage.

For testing various natural waters with NTA, surface water samples from selected streams and lakes were airmailed to Duluth in polyethylene containers and stored in the dark at 5 C prior to testing. Total and calcium hardness, alkalinity, acidity, *p*H, and conductivity were measured according to the procedures given by the American Public Health Association (1960) (Table 1). Additional experiments were conducted for comparative purposes in Lake Superior water artificially hardened with limestone (the Ca/Mg ratio was not determined) to 130 and 220 mg/liter total hardness (as $CaCO_3$). Measurements of *p*H were made in test containers for all experiments when the animals were added and 1 wk later when the medium was renewed.

An NTA formulation from Monsanto Company, supplied by Dr R. D. Swisher, was used for testing; it was 99% pure $Na_3NTA \cdot H_2O$, molecular weight 275, lot number 152049. An autoanalyzer (Technicon Corporation) was employed for NTA analyses according to the method of Thompson and Duthie (1968) with modifications as described by Arthur et al. (1974). For all tests, NTA was weighed, added to lake water, and diluted to the desired concentrations. Analytical measurements from the test containers were made at the time of addition and 1 wk later. Concentrations of NTA were slightly higher a week after addition because of a small amount of water evaporation; no degradation of NTA was evident during this time. There was good agreement between weighed amounts and analytical measurements; the coefficient of variation was 7.6% for a total of 345 determinations (with measurements at the beginning and end of weeks combined) over the range of 2–1600 mg/liter.

Additional bioassay tests were conducted on various mixtures of copper, zinc, and iron (American Chemical Society reagent grade chemicals used were $CaCl_2$, $ZnCl_2$, and $FeCl_3$; concentrations given in this paper are as the metal ions) with NTA in Lake Superior water to determine the effects of metal chelation on *Daphnia* toxicity. Combinations of NTA and Cu and NTA and Zn were tested by adding given NTA concentrations and a series of metal concentrations plus

TABLE 1. Chemical characteristics of natural waters used in NTA bioassays.

Source	Total hardness (mg/liter as $CaCO_3$)	Calcium hardness (mg/liter)	Total alkalinity (mg/liter as $CaCO_3$)	Acidity (mg/liter)	*p*H	Conductivity (μmho)
Lake Superior (Duluth, Minn.)	45	36	43	1.0	7.7	–
Ohio River (Cincinnati, Ohio)	184	138	54	3.0	7.4	453
Shayler Run (Cincinnati, Ohio)	271	240	195	6.2	8.0	648
Mississippi River (Minneapolis, Minn.)	228	150	164	–	8.5	405
Minnesota River (Minneapolis, Minn.)	438	280	232	1.8	8.1	750
Missouri River (Kansas City, Mo.)	240	182	164	6.0	7.7	640
Willamette River (Corvallis, Ore.)	22	16	28	2.1	7.2	74
Lake Erie (Cleveland, Ohio)	123	94	94	1.6	8.0	270

controls. The effect of NTA on iron toxicity was tested by the use of a series of concentrations with a constant weight ratio of iron to Na_3NTA of 1:10 (Fe/NTA = 1/2.2).

Results and discussion — The chronic toxicity of NTA to daphnids in the various waters tested and in artificially hardened Lake Superior water was largely dependent on the chemical characteristics of the test water. In the various waters the toxicity of NTA varied tenfold and showed a strong negative correlation ($r = 0.95$) between adult LC50 and water hardness (Fig. 1). Tests with Lake Superior water and two concentrations of artificially hardened Lake Superior water further substantiated this finding (Table 2 and Fig. 1). This correlation is related to the molar equivalence of NTA with calcium and magnesium, the principal hardness cations. The 16% reproductive impairment concentration (Biesinger and Christensen 1972) of NTA to daphnids in Lake Superior water was 91 mg/liter Na_3NTA; the calculated molar ratio of Ca (the major cation in Lake Superior water) to NTA was 1/1.1 at this concentration. This and higher concentrations of NTA resulted in effects on reproduction. NTA is a strong chelating agent (Ringbom 1963) and readily forms soluble complexes with calcium, magnesium, and other cations over the normal *p*H range of most natural waters. As increasing concentrations of NTA are added to water, calcium, and magnesium, ion activities are reduced in proportion to the amount of NTA added. At the calculated molar equivalence point the cationic activities are greatly reduced and free

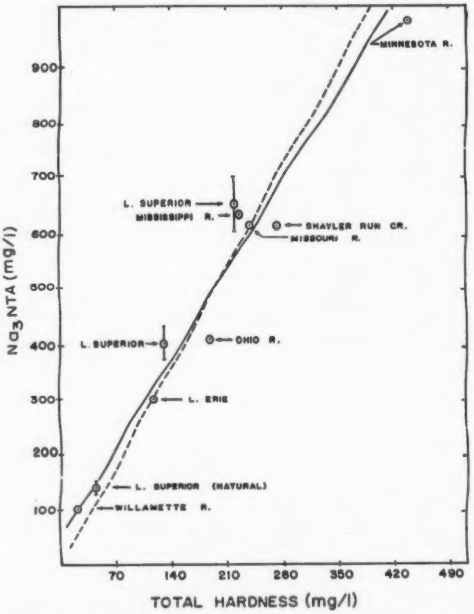

FIG. 1. Effect of total hardness on NTA toxicity to *Daphnia magna* in various natural waters and in artificially hardened Lake Superior water after 3-wk exposures. Dotted circles are the LC50 concentrations of Na_3NTA to daphnids; the vertical bars are the 95% LC50 confidence limits; the solid line is a regression line; and the dashed line is the calculated molar equivalence of total hardness and NTA.

TABLE 2. Chronic toxicity of Na_3NTA to *Daphnia magna* in natural and artificially hardened Lake Superior water after 3-wk exposures.

Source	3-wk LC50 (mg/liter Na_3NTA)	Reproductive impairment (mg/liter Na_3NTA) 50%	16%
Lake Superior (Total hardness 45 mg/liter)	145 (137–154)[a]	115	91
Lake Superior (Total hardness 130 mg/liter)	405 (378–433)	340	285
Lake Superior (Total hardness 220 mg/liter)	650 (607–696)	460	370

[a] Numbers in parentheses are 95% confidence limits.

TABLE 3. Toxicity of metal ions alone and the metal ions when chelated with NTA to *Daphnia magna* after 3-wk exposures in Lake Superior water.

Substance tested	Concentrations of metal ions in mg/liter	
	Adult 3-wk LC50	50% reproductive impairment
Copper[a]	0.044 (0.035–0.055)[b]	0.035
Copper + 2 mg/liter Na_3NTA	0.26	0.26
Zinc[a]	0.158 (0.149–0.167)	0.115
Zinc + 2 mg/liter Na_3NTA	0.48	0.45
Zinc + 8 mg/liter Na_3NTA	1.7	1.6
Iron (III)[a]	5.9 (5.2–6.7)	5.2
Iron (III) + Na_3NTA (1:10 weight basis)	9.7 (6.3–15.0)	6.4

[a] Biesinger and Christensen (1972).

[b] Numbers in parentheses are 95% LC50 confidence limits.

anionic NTA ($HNTA^=$) is formed. Our results suggest that chronic toxicity is exhibited only when equivalent or slightly excess anionic NTA is present in test solutions (Table 2).

In the various waters tested, NTA increased *p*H from 0.3 to 0.6 units above ambient control levels at concentrations near or above the 3-wk LC50s. These increases do not cause adverse effects to daphnids and were not responsible for mortality in our chronic tests. Arthur et al. (1974) also noted that chronic mortality was not caused by increased *p*H.

The toxicities of copper, zinc, and iron (III) as determined individually (Biesinger and Christensen 1972) and in combination with NTA are summarized in Table 3. Copper–NTA and zinc–NTA were considerably less toxic than the metals alone as found by Sprague (1968). A similar effect was not noted with iron (III), however, as the toxicity was only slightly decreased by a tenfold (weight basis) excess of NTA.

Copper, when chelated with 2mg/liter of Na_3NTA, and zinc, when chelated with 2 and 8 mg/liter of Na_3NTA, were essentially nontoxic. Our results suggest that these metals were toxic only when they were present in molar excess nearly equal to or greater than the LC50 of the metals alone. For example, 2 mg/liter of Na_3NTA will chelate 0.51 mg/liter zinc. Because the LC50 for zinc alone is 0.158 mg/liter, then 0.51 + 0.158 or a total of 0.67 mg/liter should approximate the LC50 in the presence of 2 mg/liter Na_3NTA. In the example given from our results the calculated LC50 was 0.48 mg/liter with 90% survival (from the dose/response line) at 0.40 mg/liter and total mortality at 0.80 mg/liter zinc.

The toxicity of Fe was not significantly changed by NTA chelation (Table 3), although a visual inspection attested to the chelation, i.e. the solutions were a much deeper yellow with NTA present, and the amount of precipitate was less. The toxic differences between iron (III) and copper–zinc chelates may be related to the differences in valence of the metals: iron (III) forms neutral–soluble chelates with NTA, whereas copper and zinc chelates are anionic (Ringbom 1963).

Concentrations of NTA which might be expected in the aquatic environment, based on possible detergent usage, would probably be less than 0.05 mg/liter (Sturm and Payne 1973). Our findings and those of others indicate that this concentration of NTA or of certain NTA–metal chelates would not be harmful to daphnids or other aquatic organisms.

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Additional Publications

The Service also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at establishments located at: Ottawa; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

Le Service publie également une série de *Bulletins*, chacun traitant sur un sujet spécifique. Les publications de l'ORP sont vendues par Information Canada à Ottawa. Les remises, payables d'avance en monnaie canadienne, doivent être établies à l'ordre du Receveur général du Canada. Les *Bulletins* sont aussi en vente chez votre libraire et dans les librairies d'Information Canada.

Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages aux établissements situés à Ottawa (Ont.) à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

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Guide to Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

EDITORIAL PRACTICES

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970), Special Publication No. 6 of the American Fisheries Society.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

Series titles should be avoided. An *abstract* is required. Authors are encouraged to submit abstracts in both English and French; those submitted in one language only are translated by the *Journal*.

Line drawings are preferably made with black india ink on white drawing or tracing paper or on coordinate paper with blue grids. All lines, symbols, letters, and numerals must be neat, and at least 1 mm high when the illustration is reduced to the appropriate size. Drawings should be grouped to facilitate comparisons. Originals must be submitted and should be less than 50 cm in the greater dimension for full-page illustrations, and proportionately smaller for those taking less than a page. Drawings must not be rolled for mailing.

Photographs should be printed on glossy paper with a wide range in tone. They should be cropped, squared, and mounted on white cardboard, with no spaces between those arranged in groups.

Galley proof is sent to the author, and he is responsible for detecting errors in printing as well as in content.

Fifty *reprints*, without covers, are supplied free. Additional copies may be ordered when the galley proof is returned. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

CHECKLIST ON MANUSCRIPTS

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the biology?

Would any of the text be clearer if condensed? Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

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Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

DISPOSITIONS GÉNÉRALES

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts.* Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Les *notes* se distinguent des *articles* par la portée et le degré d'achèvement, plutôt que par la qualité des recherches. Les notes peuvent traiter de problèmes à portée restreinte et facilement définissables; de travaux qui en corroborent d'autres; d'engins et de méthodes de pêche, ou de techniques de recherche; de découvertes dérivées d'une recherche de plus grande envergure; de progrès accomplis dans l'exécution de projets abandonnés; enfin, de résultats préliminaires assez importants pour en justifier la publication avant la solution complète du problème. L'agencement des notes peut être aussi flexible que celui des articles, sauf qu'elles ne doivent pas avoir d'en-têtes de paragraphes centrés. La longueur ne doit pas dépasser quatre pages.

On doit en général éviter les *titres en séries.* L'auteur doit fournir un *résumé.* Si possible, le résumé devra être produit en anglais et en français; le *Journal* se charge de la traduction des résumés soumis en une seule langue.

Les *dessins au trait* doivent être éxécutés de préférence à l'encre de Chine sur papier à dessin blanc, sur papier à calquer ou sur papier quadrillé bleu. Lignes, symboles, lettres et chiffres doivent être soignés et mesurer au moins 1 mm de hauteur lorsque la figure est réduite à la dimension voulue. On groupera les dessins de façon à faciliter les comparaisons. Les dessins originaux doivent accompagner le manuscrit, et ne doivent pas dépasser 50 cm dans le sens le plus long s'ils représentent une figure devant couvrir une page entière; ils seront proportionnellement plus petits pour des figures de moins d'une page. Les dessins doivent être postés à plat.

Les épreuves de *photographies* doivent être tirées sur papier glacé à gamme de ton étendue. Elles doivent être coupées, dressées et montées sur carton blanc, sans laisser d'espace entre celles qui sont disposées par groupes.

Les *épreuves en placards* sont adressées à l'auteur et il lui incombe de corriger toute erreur d'impression ou de contenu.

Cinquante *tirés à part,* sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander des tirés à part supplémentaires. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

LISTE DE CONTRÔLES POUR MANUSCRITS

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

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Guide for Typists Preparing Copy

Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and another clear copy to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).

Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send the *original* and two clear copies of each illustration with the manuscript. At the lower left of each figure and copy, give the author's name and the figure number. Also, type each caption with its figure for identification. For photographs, type this information on a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter $\underline{\mathrm{l}}$ and the numeral *one*, and between the capital letter $\overline{\underline{\mathrm{O}}}$ and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

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Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal.* Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal.*

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces), laissant toujours une marge d'au moins 2.5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation de ou des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et une seconde copie suffisamment claire au bureau du Directeur de la publication.

Tableaux: Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).
Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés.*

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par des minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Expédiez avec le manuscrit le *dessin original* et deux copies propres de chaque figure. Au coin inférieur gauche de chaque original et copie, inscrivez le nom de l'auteur et le numéro de la figure. De plus, annexez à chaque figure la légende qui lui est propre, pour fin d'identification. Dans le cas de photographies, dactylographiez l'information sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre l et le chiffre *un*, et entre la majuscule O et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le Journal (voir Appendice E). Les noms de revues sont abrégés suivant les *Biological Abstracts;* en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

Special Issue/Numéro special
Next Month/le mois prochain

As announced in an earlier issue of the *Journal*, the 19th Congress of the International Association of Limnology (SIL) is to be held in Winnipeg, Manitoba (at the Freshwater Institute of the Department of the Environment) from August 22 to 29, 1974.

This is the first occasion on which the SIL Congress will be held in Canada, and only the second time in North America. To mark this international gathering of 1500 scientists, a number of papers have been written to present in broad review the present and recent work in limnology and freshwater biology in Canada.

The May issue of the *Journal* will present these papers as a special issue, with introductory material, under the title *Limnology in Canada*.

Ainsi qu'on l'annonçait dans un numéro récent du *Journal* le 19e Congrès de la Société internationale de limnologie (SIL) aura lieu à Winnipeg, au Manitoba (à l'Institut des eaux douces du ministère de l'Environnement), du 22 au 24 août 1974.

C'est la première fois qu'un congrès de la SIL se tient au Canada, et la seconde en Amérique du Nord. À l'occasion de cette rencontre qui réunira 1500 scientifiques, on a préparé plusieurs communiqués qui donnent une vue d'ensemble des travaux canadiens actuels et récents dans les domaines de la limnologie et de la biologie des eaux douces.

En mai, le *Journal* publiera ces communiqués ainsi qu'une documentation de base dans un numéro spécial intitulé *La limnologie au Canada*.

Limnology in Canada — *Special issue prepared for the XIX Congress, International Association of Limnology (SIL)*

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